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(54) Title: INSECTICIDAL PROTEIN TOXINS FROM PHOTORHABDUS

(57) Abstract

Proteins from the genus *Photorhabdus* are toxic to insects upon exposure. *Photorhabdus luminescens* (formerly *Xenorhabdus luminescens*) have been found in mammalian clinical samples and as a bacterial symbiont of entomopathogenic nematodes of genus *Heterorhabditis*. These protein toxins can be applied to, or genetically engineered into, insect larvae food and plants for insect control.

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# INSECTICIDAL PROTEIN TOXINS FROM *PHOTORHABDUS*

## Field of the Invention

The present invention relates to toxins isolated from bacteria and the use of said toxins as insecticides.

## Background of the Invention

Many insects are widely regarded as pests to homeowners, to picnickers, to gardeners, and to farmers and others whose investments in agricultural products are often destroyed or diminished as a result of insect damage to field crops. Particularly in areas where the growing season is short, significant insect damage can mean the loss of all profits to growers and a dramatic decrease in crop yield. Scarce supply of particular agricultural products invariably results in higher costs to food processors and, then, to the ultimate consumers of food plants and products derived from those plants.

Preventing insect damage to crops and flowers and eliminating the nuisance of insect pests have typically relied on strong organic pesticides and insecticides with broad toxicities. These synthetic products have come under attack by the general population as being too harsh on the environment and on those exposed to such agents. Similarly in non-agricultural settings, homeowners would be satisfied to have insects avoid their homes or outdoor meals without needing to kill the insects.

The extensive use of chemical insecticides has raised environmental and health concerns for farmers, companies that produce the insecticides, government agencies, public interest groups, and the public in general. The development of less intrusive pest management strategies has been spurred along both by societal concern for the environment and by the development of biological tools which exploit mechanisms of insect management. Biological control agents present a promising alternative to chemical insecticides.

Organisms at every evolutionary development level have devised means to enhance their own success and survival. The use of biological molecules as tools of defense and aggression is known throughout the animal and plant kingdoms. In addition, the relatively new tools of the genetic engineer allow modifications to biological insecticides to accomplish particular solutions to particular problems.

One such agent, *Bacillus thuringiensis* (*Bt*), is an effective insecticidal agent, and is widely commercially used as such. In fact, the insecticidal agent of the *Bt* bacterium is a protein which has such limited toxicity, it can be used on human food crops on the day of harvest. To non-targeted organisms, the *Bt* toxin is a digestible non-toxic protein.

Another known class of biological insect control agents are certain genera of nematodes known to be vectors of transmission for insect-killing bacterial symbionts. Nematodes containing insecticidal bacteria invade insect larvae. The bacteria then kill the larvae. The nematodes reproduce in the larval cadaver. The nematode progeny then eat the cadaver from within. The bacteria-containing nematode progeny thus produced can then invade additional larvae.

In the past, insecticidal nematodes in the *Steinernema* and *Heterorhabditis* genera were used as insect control agents. Apparently, each genus of nematode hosts a particular species of bacterium. In nematodes of the *Heterorhabditis* genus, the symbiotic bacterium is *Photorhabdus luminescens*.

Although these nematodes are effective insect control agents, it is presently difficult, expensive, and inefficient to produce, maintain, and distribute nematodes for insect control.

It has been known in the art that one may isolate an insecticidal toxin from *Photorhabdus luminescens* that has

activity on[redacted]hen injected into Lepidopt and Coleopteran insect larvae. This has made it impossible to effectively exploit the insecticidal properties of the nematode or its bacterial symbiont. What would be useful would be a more practical, less labor-intensive wide-area delivery method of an insecticidal toxin which would retain its biological properties after delivery. It would be quite desirous to discover toxins with oral activity produced by the genus *Photorhabdus*. The isolation and use of these toxins are desirous due to efficacious reasons. Until applicants' discoveries, these toxins had not been isolated or characterized.

## Summary of the Invention

The native toxins are protein complexes that are produced and secreted by growing bacteria cells of the genus *Photorhabdus*, of interest are the proteins produced by the species *Photorhabdus luminescens*. The protein complexes, with a molecular size of approximately 1,000 kDa, can be separated by SDS-PAGE gel analysis into numerous component proteins. The toxins contain no hemolysin, lipase, type C phospholipase, or nuclease activities. The toxins exhibit significant toxicity upon exposure administration to a number of insects.

The present invention provides an easily administered insecticidal protein as well as the expression of toxin in a heterologous system.

The present invention also provides a method for delivering insecticidal toxins that are functional active and effective against many orders of insects.

Objects, advantages, and features of the present invention will become apparent from the following specification.

## Brief Description of the Drawings

Fig. 1 is an illustration of a match of cloned DNA isolates used as a part of sequence genes for the toxin of the present invention.

Fig. 2 is a map of three plasmids used in the sequencing process.

Fig. 3 is a map illustrating the inter-relationship of several partial DNA fragments.

Fig. 4 is an illustration of a homology analysis between the protein sequences of TcbAii and TcaBii proteins.

Fig. 5 is a phenogram of *Photorhabdus* strains. Relationship of *Photorhabdus* Strains was defined by rep-PCR. The upper axis of Fig. 5 measures the percentage similarity of strains based on scoring of rep-PCR products (i.e., 0.0 [no similarity] to 1.0 [100% similarity]). At the right axis, the numbers and letters indicate the various strains tested; 14=W-14, Hm=Hm, H9=H9, 7=WX-7, 1=WX-1, 2=WX-2, 88=HP88, NC-1=NC-1, 4=WX-4, 9=WX-9, 8=WX-8, 10=WX-10, WIR=WIR, 3=WX-3, 11=WX-11, 5=WX-5, 6=WX-6, 12=WX-12, x14=WX-14, 15=WX-15, Hb=Hb, B2=B2, 48 through 52=ATCC 43948 through ATCC 43952. Vertical lines separating horizontal lines indicate the degree of relatedness (as read from the extrapolated intersection of the vertical line with the upper axis) between strains or groups of strains at the base of the horizontal lines (e.g., strain W-14 is approximately 60% similar to strains H9 and Hm).

Fig. 6 is an illustration of the genomic maps of the W-14 Strain.

## Detailed Description of the Invention

The present inventions are directed to the discovery of a unique class of insecticidal protein toxins from the genus *Photorhabdus* that have oral toxicity against insects. A unique feature of *Photorhabdus* is its bioluminescence. *Photorhabdus* may be isolated from a variety of sources. One such source is nematodes, more particularly nematodes of the genus *Heterorhabditis*. Another such source is from human clinical samples from wounds, see Farmer et al. 1989 J. Clin. Microbiol. 27 pp. 1594-1600. These saprohytic strains are deposited in the American Type Culture Collection (Rockville, MD) ATCC #s 43948, 43949, 43950, 43951, and 43952, and are incorporated herein by reference. It is possible that other sources could harbor *Photorhabdus* bacteria that produce insecticidal toxins. Such sources in the environment could be either terrestrial or aquatic based.

The genus *Photorhabdus* is taxonomically defined as a member of the Family *Enterobacteriaceae*, although it has certain traits atypical of this family. For example, strains of this genus are nitrate reduction negative, yellow and red pigment producing and bioluminescent. This latter trait is otherwise unknown within the *Enterobacteriaceae*. *Photorhabdus* has only recently been described as a genus separate from the *Xenorhabdus* (Boemare et al., 1993 Int. J. Syst. Bacteriol. 43, 249-255). This differentiation is based on DNA-DNA hybridization studies, phenotypic differences (e.g., presence (*Photorhabdus*) or absence (*Xenorhabdus*) of catalase and bioluminescence) and the Family of the nematode host (*Xenorhabdus*; *Steinernematidae*, *Photorhabdus*; *Heterorhabditidae*). Comparative, cellular fatty-acid analyses (Janse et al. 1990, Lett. Appl. Microbiol 10, 131-135; Suzuki et al. 1990, J. Gen. Appl. Microbiol., 36, 393-401) support the separation of *Photorhabdus* from *Xenorhabdus*.

In order to establish that the strain collection disclosed herein was comprised of *Photorhabdus* strains, the strains were characterized based on recognized traits which define *Photorhabdus* and differentiate it from other *Enterobacteriaceae* and *Xenorhabdus* species. (Farmer, 1984 Bergey's Manual of Systemic Bacteriology Vol. 1 pp.510-511; Akhurst and Boemare 1988, J. Gen. Microbiol. 134 pp.1835-1845; Boemare et al. 1993 Int. J. Syst. Bacteriol. 43 pp.249-255, which are incorporated herein by reference). The traits studied were the following: gram stain negative rods, organism size, colony pigmentation, inclusion bodies, presence of catalase, ability to reduce nitrate, bioluminescence, dye uptake, gelatin hydrolysis, growth on selective media, growth temperature, survival under anerobic conditions and motility. Fatty acid analysis was used to confirm that the strains herein all belong to the single genus *Photorhabdus*.

Currently, the bacterial genus *Photorhabdus* is comprised of a single defined species, *Photorhabdus luminescens* (ATCC Type strain #29999, Poinar et al., 1977, Nematologica 23, 97-102). A variety of related strains have been described in the literature (e.g. Akhurst et al. 1988 J. Gen. Microbiol., 134, 1835-1845; Boemare et al. 1993 Int. J. Syst. Bacteriol. 43 pp. 249-255; Putz et al. 1990, Appl. Environ. Microbiol., 56, 181-186). Numerous

*Photorhabdus* strains have been characterized herein. Such strains are listed in Table 18 in the Examples. Because there is currently only one species (*luminescens*) defined within the genus *Photorhabdus*, the *luminescens* species traits were used to characterize the strains herein. As can be seen in Fig. 5, these strains are quite diverse. It is not unforeseen that in the future there may be other *Photorhabdus* species that will have some of the attributes of the *luminescens* species as well as some different characteristics that are presently not defined as a trait of *Photorhabdus luminescens*. However, the scope of the invention herein is to any *Photorhabdus* species or strains which produce proteins that have functional activity as insect control agents, regardless of other traits and characteristics.

Furthermore, as is demonstrated herein, the bacteria of the genus *Photorhabdus* produce proteins that have functional activity as defined herein. Of particular interest are proteins produced by the species *Photorhabdus luminescens*. The inventions herein should in no way be limited to the strains which are disclosed herein. These strains illustrate for the first time that proteins produced by diverse isolates of *Photorhabdus* are toxic upon exposure to insects. Thus, included within the inventions described herein are the strains specified herein and any mutants thereof, as well as any strains or species of the genus *Photorhabdus* that have the functional activity described herein.

There are several terms that are used herein that have a particular meaning and are as follows:

By "functional activity" it is meant herein that the protein toxins function as insect control agents in that the proteins are orally active, or have a toxic effect, or are able to disrupt or deter feeding, which may or may not cause death of the insect. When an insect comes into contact with an effective amount of toxin delivered via transgenic plant expression, formulated protein compositions(s), sprayable protein composition(s), a bait matrix or other delivery system, the results are typically death of the insect, or the insects do not feed upon the source which makes the toxins available to the insects.

The protein toxins discussed herein are typically referred to as "insecticides". By insecticides it is meant herein that the protein toxins have a "functional activity" as further defined herein and are used as insect control agents.

By the use of the term "oligonucleotides" it is meant a macromolecule consisting of a short chain of nucleotides of either RNA or DNA. Such length could be at least one nucleotide, but typically are in the range of about 10 to about 12 nucleotides. The determination of the length of the oligonucleotide is well within the skill of an artisan and should not be a limitation herein. Therefore, oligonucleotides may be less than 10 or greater than 12.

By the use of the term "toxic" or "toxicity" as used herein it is meant that the toxins produced by *Photorhabdus* have "functional activity" as defined herein.

By the use of the term "genetic material" herein, it is meant to include all genes, nucleic acid, DNA and RNA.

Fermentation broths from selected strains reported in Table 18 were used to determine the following: breadth of insecticidal toxin production by the *Photorhabdus* genus, the insecticidal spectrum of these toxins, and to provide source material to purify the toxin complexes. The strains characterized herein have been shown to have oral toxicity against a variety of insect orders. Such insect orders include but are not limited to *Coleoptera*, *Homoptera*, *Lepidoptera*, *Diptera*, *Acarina*, *Hymenoptera* and *Dictyoptera*.

As with other bacterial toxins, the rate of mutation of the bacteria in a population causes many related toxins slightly different in sequence to exist. Toxins of interest here are those which produce protein complexes toxic to a variety of insects upon exposure, as described herein. Preferably, the toxins are active against *Lepidoptera*, *Coleoptera*, *Homopotera*, *Diptera*, *Hymenoptera*, *Dictyoptera* and *Acarina*. The inventions herein are intended to capture the protein toxins homologous to protein toxins produced by the strains herein and any derivative

By the use of the term "*Photorhabdus* toxin" it is meant any protein produced by a *Photorhabdus* microorganism strain which has functional activity against insects, where the *Photorhabdus* toxin could be formulated as a sprayable composition, expressed by a transgenic plant, formulated as a bait matrix, delivered via a Baculovirus, or delivered by any other applicable host or delivery system.

strains ther as well as any protein to produced by *Photorhabdus*. These homologous proteins may differ in sequence, but do not differ in function from those toxins described herein. Homologous toxins are meant to include protein complexes of between 300 kDa to 2,000 kDa and are comprised of at least two (2) subunits, where a subunit is a peptide which may or may not be the same as the other subunit. Various protein subunits have been identified and are taught in the Examples herein. Typically, the protein subunits are between about 18 kDa to about 230 kDa; between about 160 kDa to about 230 kDa; 100 kDa to 160 kDa; about 80 kDa to about 100 kDa; and about 50 kDa to about 80 kDa.

As discussed above, some *Photorhabdus* strains can be isolated from nematodes. Some nematodes, elongated cylindrical parasitic worms of the phylum *Nematoda*, have evolved an ability to exploit insect larvae as a favored growth environment. The insect larvae provide a source of food for growing nematodes and an environment in which to reproduce. One dramatic effect that follows invasion of larvae by certain nematodes is larval death. Larval death results from the presence of, in certain nematodes, bacteria that produce an insecticidal toxin which arrests larval growth and inhibits feeding activity.

Interestingly, it appears that each genus of insect parasitic nematode hosts a particular species of bacterium, uniquely adapted for symbiotic growth with that nematode. In the interim since this research was initiated, the name of the bacterial genus *Xenorhabdus* was reclassified into the *Xenorhabdus* and the *Photorhabdus*. Bacteria of the genus *Photorhabdus* are characterized as being symbionts of *Heterorhabditus* nematodes while *Xenorhabdus* species are symbionts of the *Steinernema* species. This change in nomenclature is reflected in this specification, but in no way should a change in nomenclature alter the scope of the inventions described herein.

The peptides and genes that are disclosed herein are named according to the guidelines recently published in the Journal of Bacteriology "Instructions to Authors" p. *i-xii* (Jan. 1996), which is incorporated herein by reference. The following peptides and genes were isolated from *Photorhabdus* strain W-14.

Peptide / Gene Nomenclature
Toxin complex (Tc)

| Peptide Name | Gene Name | Patent Sequence ID# |
|---|---|---|
| *tca* genomic region | | |
| TcaA | *tcaA* | 12 |
| $TcaA_{iii}$ | *tcaA* | 4 |
| $TcaB_{i}$ | *tcaB* | 3 (19, 20) |
| $TcaB_{ii}$ | *tcaB* | 5 |
| TcaC | *tcaC* | 2 |
| *tcb* genomic region | | |
| TcbA | *tcbA* | 16 |
| $TcbA_{i}$ | *tcbA* | (pro-peptide) |
| $TcbA_{ii}$ | *tcbA* | 1 (21, 22, 23, 24) |
| $TcbA_{iii}$ | *tcbA* | 40 |
| *tcc* genomic region | | |
| TccA | *tccA* | 8 |
| TccB | *tccB* | 7 |
| *tcd* genomic region | | |
| $TcdA_{i}$ | *tcdA* | (pro-peptide) |
| $TcdA_{ii}$ | *tcdA* | 13, (38, 39 17, 18) |
| $TcdA_{iii}$ | *tcdA* | 41, (42, 43) |
| TcdB | *tcdB* | 14 |

(bracket sequence indicates internal amino acid sequence obtained by tryptic digests)

The sequences listed above are grouped by genomic region. The *tcbA* gene was expressed in *E. coli* as two protein fragments TcbA and $TcbA_{iii}$ as illustrated in the Examples. It may be beneficial to have proteolytic clippage of some sequences to obtain the higher activity of the toxins for commercial transgenic applications.

The toxins described herein are quite unique in that the toxins have functional activity, which is key to developing an insect management strategy. In developing an insect management strategy, it is possible to delay or circumvent the protein degradation process by injecting a protein directly into an organism, avoiding its digestive tract. In such cases, the protein administered to the organism will retain its function until it is denatured, non-specifically degraded, or eliminated by the immune system in higher organisms. Injection into insects

of an insecticidal toxin has potential application only in the laboratory, and then only on large insects which are easily injected. The observation that the insecticidal protein toxins herein described exhibits their toxic activity after oral ingestion or contact with the toxins permits the development of an insect management plan based solely on the ability to incorporate the protein toxins into the insect diet. Such a plan could result in the production of insect baits.

The *Photorhabdus* toxins may be administered to insects in a purified form. The toxins may also be delivered in amounts from about 1 to about 100 mg / liter of broth. This may vary upon formulation condition, conditions of the inoculum source, techniques for isolation of the toxin, and the like. The toxins may be administered as an exudate secretion or cellular protein originally expressed in a heterologous prokaryotic or eukaryotic host. Bacteria are typically the hosts in which proteins are expressed. Eukaryotic hosts could include but are not limited to plants, insects and yeast. Alternatively, the toxins may be produced in bacteria or transgenic plants in the field or in the insect by a baculovirus vector. Typically the toxins will be introduced to the insect by incorporating one or more of the toxins into the insects' feed.

Complete lethality to feeding insects is useful but is not required to achieve useful toxicity. If the insects avoid the toxin or cease feeding, that avoidance will be useful in some applications, even if the effects are sublethal. For example, if insect resistant transgenic crop plants are desired, a reluctance of insects to feed on the plants is as useful as lethal toxicity to the insects since the ultimate objective is protection of the plants rather than killing the insect.

There are many other ways in which toxins can be incorporated into an insect's diet. As an example, it is possible to adulterate the larval food source with the toxic protein by spraying the food with a protein solution, as disclosed herein. Alternatively, the purified protein could be genetically engineered into an otherwise harmless bacterium, which could then be grown in culture, and either applied to the food source or allowed to reside in the soil in an area in which insect eradication was desirable. Also, the protein could be genetically engineered directly into an insect food source. For

instance, the major food source of many insect larvae is plant material.

By incorporating genetic material that encodes the insecticidal properties of the *Photorhabdus* toxins into the genome of a plant eaten by a particular insect pest, the adult or larvae would die after consuming the food plant. Numerous members of the monocotyledonous and dictyledenous genera have been transformed. Transgenic agronmonic crops as well as fruits and vegetables are of commercial interest. Such crops include but are not limited to maize, rice, soybeans, canola, sunflower, alfalfa, sorghum, wheat, cotton, peanuts, tomatoes, potatoes, and the like. Several techniques exist for introducing foreign genetic material into plant cells, and for obtaining plants that stably maintain and express the introduced gene. Such techniques include acceleration of genetic material coated onto microparticles directly into cells(U.S. Patents 4,945,050 to Cornell and 5,141,131 to DowElanco). Plants may be transformed using *Agrobacterium* technology, see U.S. Patent 5,177,010 to University of Toledo, 5,104,310 to Texas A&M, European Patent Application 0131624B1, European Patent Applications 120516, 159418B1 and 176,112 to Schilperoot, U.S. Patents 5,149,645, 5,469,976, 5,464,763 and 4,940,838 and 4,693,976 to Schilperoot, European Patent Applications 116718, 290799, 320500 all to MaxPlanck, European Patent Applications 604662 and 627752 to Japan Tobacco, European Patent Applications 0267159, and 0292435 and U.S. Patent 5,231,019 all to Ciba Geigy, U.S. Patents 5,463,174 and 4,762,785 both to Calgene, and U.S. Patents 5,004,863 and 5,159,135 both to Agracetus. Other transformation technology includes whiskers technology, see U.S. Patents 5,302,523 and 5,464,765 both to Zeneca. Electroporation technology has also been used to transform plants, see WO 87/06614 to Boyce Thompson Institute, 5,472,869 and 5,384,253 both to Dekalb, WO9209696 and WO9321335 both to PGS. All of these transformation patents and publications are incorporated by reference. In addition to numerous technologies for transforming plants, the type of tissue which is contacted with the foreign genes may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue type I and II, hypocotyl, meristem, and the like. Almost all plant tissues may

be transfo[redacted]d during dedifferentiation [redacted]g appropriate techniques within the skill of an artisan.

Another variable is the choice of a selectable marker. The preference for a particular marker is at the discretion of the artisan, but any of the following selectable markers may be used along with any other gene not listed herein which could function as a selectable marker. Such selectable markers include but are not limited to aminoglycoside phosphotransferase gene of transposon Tn5 (Aph II) which encodes resistance to the antibiotics kanamycin, neomycin and G418, as well as those genes which code for resistance or tolerance to glyphosate; hygromycin; methotrexate; phosphinothricin (bialophos); imidazolinones, sulfonylureas and triazolopyrimidine herbicides, such as chlorosulfuron; bromoxynil, dalapon and the like.

In addition to a selectable marker, it may be desirous to use a reporter gene. In some instances a reporter gene may be used without a selectable marker. Reporter genes are genes which are typically not present or expressed in the recipient organism or tissue. The reporter gene typically encodes for a protein which provides for some phenotypic change or enzymatic property. Examples of such genes are provided in K. Weising et al. Ann. Rev. Genetics, 22, 421 (1988), which is incorporated herein by reference. A preferred reporter gene is the glucuronidase (GUS) gene.

Regardless of transformation technique, the gene is preferably incorporated into a gene transfer vector adapted to express the *Photorhabdus* toxins in the plant cell by including in the vector a plant promoter. In addition to plant promoters, promoters from a variety of sources can be used efficiently in plant cells to express foreign genes. For example, promoters of bacterial origin, such as the octopine synthase promoter, the nopaline synthase promoter, the mannopine synthase promoter; promoters of viral origin, such as the cauliflower mosaic virus (35S and 19S)and the like may be used. Plant promoters include, but are not limited to ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu), beta-conglycinin promoter, phaseolin promoter, ADH promoter, heat-shock promoters and tissue specific promoters. Promoters may also contain certain enhancer sequence elements that may improve the transcription efficiency. Typical enhancers include but are not limited to Adh-intron 1 and

Adh-intron 6. Constitutive promoters may be used. Constitutive promoters direct continuous gene expression in all cells types and at all times (e.g., actin, ubiquitin, CaMV 35S). Tissue specific promoters are responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (e.g., zein, oleosin, napin, ACP) and these promoters may also be used. Promoters may also be are active during a certain stage of the plants' development as well as active in plant tissues and organs. Examples of such promoters include but are not limited to pollen-specific, embryo specific, corn silk specific, cotton fiber specific, root specific, seed endosperm specific promoters and the like.

Under certain circumstances it may be desirable to use an inducible promoter. An inducible promoter is responsible for expression of genes in response to a specific signal, such as: physical stimulus (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites; and stress. Other desirable transcription and translation elements that function in plants may be used. Numerous plant-specific gene transfer vectors are known to the art.

In addition, it is known that to obtain high expression of bacterial genes in plants it is preferred to reengineer the bacterial genes so that they are more efficiently expressed in the cytoplasm of plants. Maize is one such plant where it is preferred to reengineer the bacterial gene(s) prior to transformation to increase the expression level of the toxin in the plant. One reason for the reengineering is the very low G+C content of the native bacterial gene(s) (and consequent skewing towards high A+T content). This results in the generation of sequences mimicking or duplicating plant gene control sequences that are known to be highly A+T rich. The presence of some A+T-rich sequences within the DNA of the gene(s) introduced into plants (e.g., TATA box regions normally found in gene promoters) may result in aberrant transcription of the gene(s). On the other hand, the presence of other regulatory sequences residing in the transcribed mRNA (e.g., polyadenylation signal sequences (AAUAAA), or sequences complementary to small nuclear RNAs involved in pre-mRNA splicing) may lead to RNA instability. Therefore, one goal in the design of reengineered bacterial

gene(s), more preferably referred to as plant optimized gene(s), is to generate a DNA sequence having a higher G+C content, and preferably one close to that of plant genes coding for metabolic enzymes. Another goal in the design of the plant optimized gene(s) is to generate a DNA sequence that not only has a higher G+C content, but by modifying the sequence changes, should be made so as to not hinder translation.

An example of a plant that has a high G+C content is maize. The table below illustrates how high the G+C content is in maize. As in maize, it is thought that G+C content in other plants is also high.

Table 1
Compilation of G+C contents of protein coding regions of maize genes

| Protein Class[a] | Range %G+C | Mean %G+C[b] |
|---|---|---|
| Metabolic Enzymes (40) | 44.4-75.3 | 59.0 (8.0) |
| Storage Proteins | | |
| Group I (23) | 46.0-51.9 | 48.1 (1.3) |
| Group II (13) | 60.4-74.3 | 67.5 (3.2) |
| Group I + II (36) | 46.0-74.3 | 55.1 (9.6)[c] |
| Structural Proteins (18) | 48.6-70.5 | 63.6 (6.7) |
| Regulatory Proteins (5) | 57.2-68.9 | 62.0 (4.9) |
| Uncharacterized Proteins (9) | 41.5-70.3 | 64.3 (7.2) |
| **All Proteins (108)** | **44.4-75.3** | **60.8 (5.2)** |

[a] Number of genes in class given in parentheses.
[b] Standard deviations given in parentheses.
[c] Combined groups mean ignored in calculation of overall mean.

For the data in Table 1, coding regions of the genes were extracted from GenBank (Release 71) entries, and base compositions were calculated using the MacVector™ program (IBI, New Haven, CT). Intron sequences were ignored in the

calculations. Group I and II storage protein gene sequences were distinguished by their marked difference in base composition.

Due to the plasticity afforded by the redundancy of the genetic code (i.e., some amino acids are specified by more than one codon), evolution of the genomes of different organisms or classes or organisms has resulted in differential usage of redundant codons. This "codon bias" is reflected in the mean base composition of protein coding regions. For example, organisms with relatively low G+C contents utilize codons having A or T in the third position of redundant codons, whereas those having higher G+C contents utilize codons having G or C in the third position. It is thought that the presence of "minor" codons within a gene's mRNA may reduce the absolute translation rate of that mRNA, especially when the relative abundance of the charged tRNA corresponding to the minor codon is low. An extension of this is that the diminution of translation rate by individual minor codons would be at least additive for multiple minor codons. Therefore, mRNAs having high relative contents of minor codons would have correspondingly low translation rates. This rate would be reflected by the synthesis of low levels of the encoded protein.

In order to reengineer the bacterial gene(s), the codon bias of the plant is determined. The codon bias is the statistical codon distribution that the plant uses for coding its proteins. After determining the bias, the percent frequency of the codons in the gene(s) of interest is determined. The primary codons preferred by the plant should be determined as well as the second and third choice of preferred codons. The amino acid sequence of the protein of interest is reverse translated so that the resulting nucleic acid sequence codes for the same protein as the native bacterial gene, but the resulting nucleic acid sequence corresponds to the first preferred codons of the desired plant. The new sequence is analyzed for restriction enzyme sites that might have been created by the modification. The identified sites are further modified by replacing the codons with second or third choice preferred codons. Other sites in the sequence which could affect the transcription or translation of the gene of interest are the exon:intron 5' or 3' junctions, poly A addition signals, or RNA polymerase termination signals. Th sequence is

further analyzed and modified to reduce the frequency of TA or GC doublets. In addition to the doublets, G or C sequence blocks that have more than about four residues that are the same can affect transcription of the sequence. Therefore, these blocks are also modified by replacing the codons of first or second choice, etc. with the next preferred codon of choice. It is preferred that the plant optimized gene(s) contains about 63% of first choice codons, between about 22% to about 37% second choice codons, and between 15% and 0% third choice codons, wherein the total percentage is 100%. Most preferred the plant optimized gene(s) contain about 63% of first choice codons, at least about 22% second choice codons, about 7.5% third choice codons, and about 7.5% fourth choice codons, wherein the total percentage is 100%. The method described above enables one skilled in the art to modify gene(s) that are foreign to a particular plant so that the genes are optimally expressed in plants. The method is further illustrated in pending provisional application U.S. 60/005,405 filed on October 13, 1995, which is incorporated herein by reference.

Thus, in order to design plant optimized gene(s) the amino acid sequence of the toxins are reverse translated into a DNA sequence, utilizing a nonredundant genetic code established from a codon bias table compiled for the gene DNA sequence for the particular plant being transformed. The resulting DNA sequence, which is completely homogeneous in codon usage, is further modified to establish a DNA sequence that, besides having a higher degree of codon diversity, also contains strategically placed restriction enzyme recognition sites, desirable base composition, and a lack of sequences that might interfere with transcription of the gene, or translation of the product mRNA.

It is theorized that bacterial genes may be more easily expressed in plants if the bacterial genes are expressed in the plastids. Thus, it may be possible to express bacterial genes in plants, without optimizing the genes for plant expression, and obtain high express of the protein. See U.S. Patent Nos. 4,762,785; 5,451,513 and 5,545,817, which are incorporated herein by reference.

One of the issues regarding commercial exploiting transgenic plants is resistance management. This is of particular concern with *Bacillus thuringiensis* toxins. There are numerous companies commerically exploiting *Bacillus thuringiensis* and there has been much concern about *Bt* toxins becoming resistant. One strataegy for insect resistant management would be to combine the toxins produced by *Photorhabdus* with toxins such as *Bt*, vegetative insect proteins (Ciba Geigy) or other toxins. The combinations could be formulated for a sprayable application or could be molecular combinations. Plants could be transformed with *Photorhabdus* genes that produce insect toxins and other insect toxin genes such as *Bt* as with other insect toxin genes such as *Bt*.

European Patent Application 0400246A1 describes transformation of 2 *Bt* in a plant, which could be any 2 genes. Another way to produce a transgenic plant that contains more than one insect resistant gene would be to produce two plants, with each plant containing an insect resistant gene. These plants would be backcrossed using traditional plant breeding techniques to produce a plant containing more than one insect resistant gene.

In addition to producing a transformed plant containing plant optimized gene(s), there are other delivery systems where it may be desirable to reengineer the bacterial gene(s). Along the same lines, a genetically engineered, easily isolated protein toxin fusing together both a molecule attractive to insects as a food source and the insecticidal activity of the toxin may be engineered and expressed in bacteria or in eukaryotic cells using standard, well-known techniques. After purification in the laboratory such a toxic agent with "built-in" bait could be packaged inside standard insect trap housings.

Another delivery scheme is the incorporation of the genetic material of toxins into a baculovirus vector. Baculoviruses infect particular insect hosts, including those desirably targeted with the *Photorhabdus* toxins. Infectious baculovirus harboring an expression construct for the *Photorhabdus* toxins could be introduced into areas of insect infestation to thereby intoxicate or poison infected insects.

Transfer of the insecticidal properties requires nucleic acid sequences encoding the coding the amino acid sequences for the *Photorhabdus* toxins integrated into a protein expression vector appropriate to the host in which the vector will reside. One way to obtain a nucleic acid sequence encoding a protein with insecticidal properties is to isolate the native genetic material which produces the toxins from *Photorhabdus*, using information deduced from the toxin's amino acid sequence, large portions of which are set forth below. As described below, methods of purifying the proteins responsible for toxin activity are also disclosed.

Using N-terminal amino acid sequence data, such as set forth below, one can construct oligonucleotides complementary to all, or a section of, the DNA bases that encode the first amino acids of the toxin. These oligonucleotides can be radiolabeled and used as molecular probes to isolate the genetic material from a genomic genetic library built from genetic material isolated from strains of *Photorhabdus*. The genetic library can be cloned in plasmid, cosmid, phage or phagemid vectors. The library could be transformed into *Escherichia coli* and screened for toxin production by the transformed cells using antibodies raised against the toxin or direct assays for insect toxicity.

This approach requires the production of a battery of oligonucleotides, since the degenerate genetic code allows an amino acid to be encoded in the DNA by any of several three-nucleotide combinations. For example, the amino acid arginine can be encoded by nucleic acid triplets CGA, CGC, CGG, CGT, AGA, and AGG. Since one cannot predict which triplet is used at those positions in the toxin gene, one must prepare oligonucleotides with each potential triplet represented. More than one DNA molecule corresponding to a protein subunit may be necessary to construct a sufficient number of oligonucleotide probes to recover all of the protein subunits necessary to achieve oral toxicity.

From the amino acid sequence of the purified protein, genetic materials responsible for the production of toxins can readily be isolated and cloned, in whole or in part, into an expression vector using any of several techniques well-known to one skilled in the art of molecular biology. A typical expression vector is a DNA plasmid, though other transfer means

including, but not limited to, cosmids, phagemids and phage are also envisioned. In addition to features required or desired for plasmid replication, such as an origin of replication and antibiotic resistance or other form of a selectable marker such as the *bar* gene of *Streptomyces hygroscopicus* or *viridochromogenes*, protein expression vectors normally additionally require an expression cassette which incorporates the cis-acting sequences necessary for transcription and translation of the gene of interest. The cis-acting sequences required for expression in prokaryotes differ from those required in eukaryotes and plants.

A eukaryotic expression cassette requires a transcriptional promoter upstream (5') to the gene of interest, a transcriptional termination region such as a poly-A addition site, and a ribosome binding site upstream of the gene of interest's first codon. In bacterial cells, a useful transcriptional promoter that could be included in the vector is the T7 RNA Polymerase-binding promoter. Promoters, as previously described herein, are known to efficiently promote transcription of mRNA. Also upstream from the gene of interest the vector may include a nucleotide sequence encoding a signal sequence known to direct a covalently linked protein to a particular compartment of the host cells such as the cell surface.

Insect viruses, or baculoviruses, are known to infect and adversely affect certain insects. The affect of the viruses on insects is slow, and viruses do not stop the feeding of insects. Thus viruses are not viewed as being useful as insect pest control agents. Combining the *Photorhabdus* toxins genes into a baculovirus vector could provide an efficient way of transmitting the toxins while increasing the lethality of the virus. In addition, since different baculoviruses are specific to different insects, it may be possible to use a particular toxin to selectively target particularly damaging insect pests. A particularly useful vector for the toxins genes is the nuclear polyhedrosis virus. Transfer vectors using this virus have been described and are now the vectors of choice for transferring foreign genes into insects. The virus-toxin gene recombinant may be constructed in an orally transmissible form. Baculoviruses normally infect insect victims through the mid-gut intestinal mucosa. The toxin gene inserted behind a strong viral coat

protein prom would be expressed and sh rapidly kill the infected insect.

In addition to an insect virus or baculovirus or transgenic plant delivery system for the protein toxins of the present invention, the proteins may be encapsulated using *Bacillus thuringiensis* encapsulation technology such as but not limited to U.S. Patent Nos. 4,695,455; 4,695,462; 4,861,595 which are all incorporated herein by reference. Another delivery system for the protein toxins of the present invention is formulation of the protein into a bait matrix, which could then be used in above and below ground insect bait stations. Examples of such technology include but are not limited to PCT Patent Application WO 93/23998, which is incorporated herein by reference.

As is described above, it might become necessary to modify the sequence encoding the protein when expressing it in a non-native host, since the codon preferences of other hosts may differ from that of *Photorhabdus*. In such a case, translation may be quite inefficient in a new host unless compensating modifications to the coding sequence are made. Additionally, modifications to the amino acid sequence might be desirable to avoid inhibitory cross-reactivity with proteins of the new host, or to refine the insecticidal properties of the protein in the new host. A genetically modified toxin gene might encode a toxin exhibiting, for example, enhanced or reduced toxicity, altered insect resistance development, altered stability, or modified target species specificity.

In addition to the *Photorhabdus* genes encoding the toxins, the scope of the present invention is intended to include related nucleic acid sequences which encode amino acid biopolymers homologous to the toxin proteins and which retain the toxic effect of the *Photorhabdus* proteins in insect species after oral ingestion.

For instance, the toxins used in the present invention seem to first inhibit larval feeding before death ensues. By manipulating the nucleic acid sequence of *Photorhabdus* toxins or its controlling sequences, genetic engineers placing the toxin gene into plants could modulate its potency or its mode of action to, for example, keep the eating-inhibitory activity while eliminating the absolute toxicity to the larvae. This change could permit the transformed plant to survive until harvest

without h[redacted]ng the unnecessarily dramat effect on the ecosystem of wiping out all target insects. All such modifications of the gene encoding the toxin, or of the protein encoded by the gene, are envisioned to fall within the scope of the present invention.

Other envisioned modifications of the nucleic acid include the addition of targeting sequences to direct the toxin to particular parts of the insect larvae for improving its efficiency.

Strains ATCC 55397, 43948, 43949, 43950, 43951, 43952 have been deposited in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 USA. Amino acid and nucleotide sequence data for the W-14 native toxin (ATCC 55397) is presented below. Isolation of the genomic DNA for the toxins from the bacterial hosts is also exemplified herein.

Standard and molecular biology techniques were followed and taught in the specification herein. Additional information may be found in Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989), Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, which is incorporated herein by reference.

The following abbreviations are used throughout the Examples: Tris = tris (hydroxymethyl) amino methane; SDS = sodium dodecyl sulfate; EDTA = ethylenediaminetetraacetic acid, IPTG = isopropylthio-B-galactoside, X-gal = 5-bromo-4-chloro-3-indoyl-B-D-galactoside, CTAB = cetyltrimethylammonium bromide; kbp = kilobase pairs; dATP, dCTP, dGTP, dTTP, I = 2'-deoxynucleoside 5'-triphosphates of adenine, cytosine, guanine, thymine, and inosine, respectively; ATP = adenosine 5' triphosphate.

## Example 1

## Purification of toxin from *P. luminescens* and Demonstration of toxicity after oral delivery of purified toxin

The insecticidal protein toxin of the present invention was purified from *P. luminescens* strain W-14, ATCC Accession Number 55397. Stock cultures of *P. luminescens* were maintained on petri dishes containing 2% Proteose Peptone No. 3 (i.e., PP3, Difco Laboratories, Detroit MI) in 1.5% agar, incubated at 25°C and transferred weekly. Colonies of the primary form of the bacteria were inoculated into 200 ml of PP3 broth supplemented with 0.5%

polyoxyethylene sorbitan mono-stearate (Tween 60, Sigma Chemical Company, St. Louis MO) in a one liter flask. The broth cultures were grown for 72 hours at 30°C on a rotary shaker. The toxin proteins can be recovered from cultures grown in the presence or absence of Tween; however, the absence of Tween can affect the form of the bacteria grown and the profile of proteins produced by the bacteria. In the absence of Tween, a variant shift occurs insofar as the molecular weight of at least one identified toxin subunit shifts from about 200 kDa to about 185 kDa.

The 72 hour cultures were centrifuged at 10,000 x g for 30 minutes to remove cells and debris. The supernatant fraction that contained the insecticidal activity was decanted and brought to 50 mM $K_2HPO_4$ by adding an appropriate volume of 1.0 M $K_2HPO_4$. The pH was adjusted to 8.6 by adding potassium hydroxide. This supernatant fraction was then mixed with DEAE-Sephacel (Pharmacia LKB Biotechnology) which had been equilibrated with 50 mM $K_2HPO_4$. The toxic activity was adsorbed to the DEAE resin. This mixture was then poured into a 2.6 x 40 cm column and washed with 50 mM $K_2HPO_4$ at room temperature at a flow rate of 30 ml/hr until the effluent reached a steady baseline UV absorbance at 280 nm. The column was then washed with 150 mM KCl until the effluent again reached a steady 280 nm baseline. Finally the column was washed with 300 mM KCl and fractions were collected.

Fractions containing the toxin were pooled and filter sterilized using a 0.2 micron pore membrane filter. The toxin was then concentrated and equilibrated to 100 mM $KPO_4$, pH 6.9, using an ultrafiltration membrane with a molecular weight cutoff of 100 kDa at 4°C (Centriprep 100, Amicon Division-W.R. Grace and Company). A 3 ml sample of the toxin concentrate was applied to the top of a 2.6 x 95 cm Sephacryl S-400 HR gel filtration column (Pharmacia LKB Biotechnology). The eluent buffer was 100 mM $KPO_4$, pH 6.9, which was run at a flow rate of 17 ml/hr, at 4°C. The effluent was monitored at 280 nm.

Fractions were collected and tested for toxic activity. Toxicity of chromatographic fractions was examined in a biological assay using *Manduca sexta* larvae. Fractions were either applied directly onto the insect diet (Gypsy moth wheat germ diet, ICN Biochemicals Division - ICN Biomedicals, Inc.) or administered by intrahemocelic injection of a 5 µl sample through the first proleg of 4th or 5th instar larva using a 30 gauge

needle. weight of each larva within treatment group was recorded at 24 hour intervals. Toxicity was presumed if the insect ceased feeding and died within several days of consuming treated insect diet or if death occurred within 24 hours after injection of a fraction.

The toxic fractions were pooled and concentrated using the Centriprep-100 and were then analyzed by HPLC using a 7.5 mm x 60 cm TSK-GEL G-4000 SW gel permeation column with 100 mM potassium phosphate, pH 6.9 eluent buffer running at 0.4 ml/min. This analysis revealed the toxin protein to be contained within a single sharp peak that eluted from the column with a retention time of approximately 33.6 minutes. This retention time corresponded to an estimated molecular weight of 1,000 kDa. Peak fractions were collected for further purification while fractions not containing this protein were discarded. The peak eluted from the HPLC absorbs UV light at 218 and 280 nm but did not absorb at 405 nm. Absorbance at 405 nm was shown to be an attribute of xenorhabdin antibiotic compounds.

Electrophoresis of the pooled peak fractions in a non-denaturing agarose gel (Metaphor Agarose, FMC BioProducts) showed that two protein complexes are present in the peak. The peak material, buffered in 50 mM Tris-HCl, pH 7.0, was separated on a 1.5% agarose stacking gel buffered with 100 mM Tris-HCl at pH 7.0 and 1.9% agarose resolving gel buffered with 200 mM Tris-borate at pH 8.3 under standard buffer conditions (anode buffer 1M Tris-HCl, pH 8.3; cathode buffer 0.025 M Tris, 0.192 M glycine). The gels were run at 13 mA constant current at 15°C until the phenol red tracking dye reached the end of the gel. Two protein bands were visualized in the agarose gels using Coomassie brilliant blue staining.

The slower migrating band was referred to as "protein band 1" and faster migrating band was referred to as "protein band 2." The two protein bands were present in approximately equal amounts. The Coomassie stained agarose gels were used as a guide to precisely excise the two protein bands from unstained portions of the gels. The excised pieces containing the protein bands were macerated and a small amount of sterile water was added. As a control, a portion of the gel that contained no protein was also excised and treated in the same manner as the gel pieces containing the protein. Protein was recovered from the gel

pieces by electroelution into 100 mM Tris-borate pH 8.3, at 100 volts (constant voltage) for two hours. Alternatively, protein was passively eluted from the gel pieces by adding an equal volume of 50 mM Tris-HCl, pH 7.0, to the gel pieces, then incubating at 30°C for 16 hours. This allowed the protein to diffuse from the gel into the buffer, which was then collected.

Results of insect toxicity tests using HPLC-purified toxin (33.6 min. peak) and agarose gel purified toxin demonstrated toxicity of the extracts. Injection of 1.5 µg of the HPLC purified protein kills within 24 hours. Both protein bands 1 and 2, recovered from agarose gels by passive elution or electroelution, were lethal upon injection. The protein concentration estimated for these samples was less than 50 ng/larva. A comparison of the weight gain and the mortality between the groups of larvae injected with protein bands 1 or 2 indicate that protein band 1 was more toxic by injection delivery.

When HPLC-purified toxin was applied to larval diet at a concentration of 7.5 µg/larva, it caused a halt in larval weight gain (24 larvae tested). The larvae begin to feed, but after consuming only a very small portion of the toxin treated diet they began to show pathological symptoms induced by the toxin and the larvae cease feeding. The insect frass became discolored and most larva showed signs of diarrhea. Significant insect mortality resulted when several 5 µg toxin doses were applied to the diet over a 7-10 day period.

Agarose-separated protein band 1 significantly inhibited larval weight gain at a dose of 200 ng/larva. Larvae fed similar concentrations of protein band 2 were not inhibited and gained weight at the same rate as the control larvae. Twelve larvae were fed eluted protein and 45 larvae were fed protein-containing agarose pieces. These two sets of data indicate that protein band 1 was orally toxic to *Manduca sexta*. In this experiment it appeared that protein band 2 was not toxic to *Manduca sexta*.

Further analysis of protein bands 1 and 2 by SDS-PAGE under denaturing conditions showed that each band was composed of several smaller protein subunits. Proteins were visualized by Coomassie brilliant blue staining followed by silver staining to achieve maximum sensitivity.

The protein subunits in the two bands were very similar. Protein band 1 contains 8 protein subunits of 25.1, 56.2, 60.8, 65.6, 166, 171, 184 and 208 kDa. Protein band 2 had an identical profile except that the 25.1, 60.8, and 65.6 kDa proteins were not present. The 56.2, 60.8, 65.6, and 184 kDa proteins were present in the complex of protein band 1 at approximately equal concentrations and represent 80% or more of the total protein content of that complex.

The native HPLC-purified toxin was further characterized as follows. The toxin was heat labile in that after being heated to 60°C for 15 minutes it lost its ability to kill or to inhibit weight gain when injected or fed to *M. sexta* larvae. Assays were designed to detect lipase, type C phospholipase, nuclease or red blood cell hemolysis activities and were performed with purified toxin. None of these activities were present. Antibiotic zone inhibition assays were also done and the purified toxin failed to inhibit growth of Gram-negative or -positive bacteria, yeast or filamentous fungi, indicating that the toxic is not a xenorhabdin antibiotic.

The native HPLC-purified toxin was tested for ability to kill insects other than *Manduca sexta*. Table 2 lists insects killed by the HPLC-purified *P. luminescens* toxin in this study.

Table 2

Insects Killed by *P. luminescens* Toxin

| Common Name | Order | Genus and species | Route of Delivery |
|---|---|---|---|
| Tobacco horn worm | Lepidoptera | *Manduca sexta* | Oral and injected |
| Mealworm | Coleoptera | *Tenebrio molitor* | Oral |
| Pharaoh ant | Hymenoptera | *Monomorium pharoanis* | Oral |
| German cockroach | Dictyoptera | *Blattella germanica* | Oral and injected |
| Mosquito | Diptera | *Aedes aegypti* | Oral |

Example 2

Insecticide Utility

The *Photorhabdus luminescens* utility and toxicity were further characterized. *Photorhabdus luminescens* (strain W-14) culture broth was produced as follows. The production medium was 2% Bacto Proteose Peptone® Number 3 (PP3, Difco Laboratories, Detroit, Michigan) in Milli-Q® deionized water. Seed culture flasks consisted of 175 ml medium placed in a 500 ml tribaffled flask with a Delong neck, covered with a Kaput and autoclaved for 20 minutes, T=250°F. Production flasks consisted of 500 mls in a 2.8 liter 500 ml tribaffled flask with a Delong neck, covered by a Shin-etsu silicon foam closure. These were autoclaved for 45 minutes, T=250°F. The seed culture was incubated at 28°C at 150 rpm in a gyrotory shaking incubator with a 2 inch throw. After 16 hours of growth, 1% of the seed culture was placed in the production flask which was allowed to grow for 24 hours before harvest. Production of the toxin appears to be during log phase growth. The microbial broth was transferred to a 1L centrifuge bottle and the cellular biomass was pelleted (30 minutes at 2500 RPM at 4°C, [R.C.F. = ~1600] HG-4L Rotor RC3 Sorval centrifuge, Dupont, Wilmington, Delaware). The primary broth was chilled at 4°C for 8 - 16 hours and recentrifuged at least 2 hours (conditions above) to further clarify the broth by removal of a putative mucopolysaccharide which precipitated upon standing. (An alternative processing method combined both steps and involved the use of a 16 hour clarification centrifugation, same conditions as above.) This broth was then stored at 4°C prior to bioassay or filtration.

*Photorhabdus* culture broth and protein toxin(s) purified from this broth showed activity (mortality and/or growth inhibition, reduced adult emergence) against a number of insects. More specifically, the activity is seen against corn rootworm (larvae and adult), Colorado potato beetle, and turf grubs, which are members of the insect order *Coleoptera*. Other members of the *Coleoptera* include wireworms, pollen beetles, flea beetles, seed beetles and weevils. Activity has also been observed against aster leafhopper, which is a member of the order, *Homoptera*. Other members of the *Homoptera* include planthoppers, pear pysIla, apple sucker, scale insects, whiteflies, and spittle bugs, as

well as n ·ous host specific aphid sp es. The broth and purified fractions are also active against beet armyworm, cabbage looper, black cutworm, tobacco budworm, European corn borer, corn earworm, and codling moth, which are members of the order *Lepidoptera*. Other typical members of this order are clothes moth, Indian mealmoth, leaf rollers, cabbage worm, cotton bollworm, bagworm, Eastern tent caterpillar, sod webworm, and fall armyworm. Activity is also seen against fruitfly and mosquito larvae, which are members of the order *Diptera*. Other members of the order *Diptera* are pea midge, carrot fly, cabbage root fly, turnip root fly, onion fly, crane fly, house fly, and various mosquito species. Activity is seen against carpenter ant and Argentine ant, which are members of the order that also includes fire ants, oderous house ants, and little black ants.

The broth/fraction is useful for reducing populations of insects and were used in a method of inhibiting an insect population. The method may comprise applying to a locus of the insect an effective insect inactivating amount of the active described. Results are reported in Table 3.

Activity against corn rootworm larvae was tested as follows. *Photorhabdus* culture broth (filter sterilized, cell-free) or purified HPLC fractions were applied directly to the surface (~1.5 $cm^2$) of 0.25 ml of artificial diet in 30 µl aliquots following dilution in control medium or 10 mM sodium phosphate buffer, pH 7.0, respectively. The diet plates were allowed to air-dry in a sterile flow-hood and the wells were infested with single, neonate *Diabrotica undecimpunctata howardi* (Southern corn rootworm, SCR) hatched from sterilized eggs, with second instar SCR grown on artificial diet or with second instar *Diabrotica virgifera virgifera* (Western corn rootworm, WCR) reared on corn seedlings grown in Metromix®. Second instar larvae were weighed prior to addition to the diet. The plates were sealed, placed in a humidified growth chamber and maintained at 27°C for the appropriate period (4 days for neonate and adult SCR, 2-5 days for WCR larvae, 7-14 days for second instar SCR). Mortality and weight determinations were scored as indicated. Generally, 16 insects per treatment were used in all studies. Control mortalities were as follows: neonate larvae, <5%, adult beetles, 5%.

Activity against Colorado potato beet as tested as follows. *Photorhabdus* culture broth or control medium was applied to the surface (~2.0 $cm^2$) of 1.5 ml of standard artificial diet held in the wells of a 24-well tissue culture plate. Each well received 50 µl of treatment and was allowed to air dry. Individual second instar Colorado potato beetle (*Leptinotarsa decemlineata*, CPB) larvae were then placed onto the diet and mortality was scored after 4 days. Ten larvae per treatment were used in all studies. Control mortality was 3.3%.

Activity against Japanese beetle grubs and beetles was tested as follows. Turf grubs (*Popillia japonica*, 2-3rd instar) were collected from infested lawns and maintained in the laboratory in soil/peat mixture with carrot slices added as additional diet. Turf beetles were pheromone-trapped locally and maintained in the laboratory in plastic containers with maple leaves as food. Following application of undiluted *Photorhabdus* culture broth or control medium to corn rootworm artificial diet (30 µl/1.54 $cm^2$, beetles) or carrot slices (larvae), both stages were placed singly in a diet well and observed for any mortality and feeding. In both cases there was a clear reduction in the amount of feeding (and feces production) observed.

Activity against mosquito larvae was tested as follows. The assay was conducted in a 96-well microtiter plate. Each well contained 200 µl of aqueous solution (*Photorhabdus* culture broth, control medium or $H_20$) and approximately 20, 1-day old larvae (*Aedes aegypti*). There were 6 wells per treatment. The results were read at 2 hours after infestation and did not change over the three day observation period. No control mortality was seen.

Activity against fruitflies was tested as follows. Purchased *Drosophila melanogaster* medium was prepared using 50% dry medium and a 50% liquid of either water, control medium or *Photorhabdus* culture broth. This was accomplished by placing 8.0 ml of dry medium in each of 3 rearing vials per treatment and adding 8.0 ml of the appropriate liquid. Ten late instar *Drosophila melanogaster* maggots were then added to each vial. The vials were held on a laboratory bench, at room temperature, under fluorescent ceiling lights. Pupal or adult counts were made after 3, 7 and 10 days of exposure. Incorporation of *Photorhabdus* culture broth into the diet media for fruitfly

maggots [redacted]sed a slight (17%) but sign[redacted]cant reduction in day-10 adult emergence as compared to water and control medium (3% reduction).

Activity against aster leafhopper was tested as follows. The ingestion assay for aster leafhopper (*Macrosteles severini*) is designed to allow ingestion of the active without other external contact. The reservoir for the active/"food" solution is made by making 2 holes in the center of the bottom portion of a 35 x 10 mm Petri dish. A 2 inch Parafilm M® square is placed across the top of the dish and secured with an "O" ring. A 1 oz. plastic cup is then infested with approximately 7 leafhoppers and the reservoir is placed on top of the cup, Parafilm down. The test solution is then added to the reservoir through the holes. In tests using undiluted *Photorhabdus* culture broth, the broth and control medium were dialyzed against water to reduce control mortality. Mortality is reported at day 2 where 26.5% control mortality was seen. In the tests using purified fractions (200 mg protein/ml ) a final concentration of 5% sucrose was used in all treatments to improve survivability of the aster leafhoppers. The assay was held in an incubator at 28°C, 70% RH with a 16/8 photoperiod. The assay was graded for mortality at 72 hours. Control mortality was 5.5%.

Activity against Argentine ants was tested as follows. A 1.5 ml aliquot of 100% *Photorhabdus* culture broth, control medium or water was pipetted into 2.0 ml clear glass vials. The vials were plugged with a piece of cotton dental wick that was moistened with the appropriate treatment. Each vial was placed into a separate 60x16mm Petri dish with 8 to 12 adult Argentine ants (*Linepithema humile*). There were three replicates per treatment. Bioassay plates were held on a laboratory bench, at room temperature under fluorescent ceiling lights. Mortality readings were made after 5 days of exposure. Control mortality was 24%.

Activity against carpenter ant was tested as follows. Black carpenter ant workers (*Camponotus pennsylvanicus*) were collected from trees on DowElanco property in Indianapolis, IN. Tests with *Photorhabdus* culture broth were performed as follows. Each plastic bioassay container (7 1/8" x 3") held fifteen workers, a paper harborage and 10 ml of broth or control media in a plastic shot glass. A cotton wick delivered the treatment to the ants

through a hole in the shot glass lid. All treatments contained 5% sucrose. Bioassays were held in the dark at room temperature and graded at 19 days. Control mortality was 9%. Assays delivering purified fractions utilized artificial ant diet mixed with the treatment (purified fraction or control solution) at a rate of 0.2 ml treatment/2.0 g diet in a plastic test tube. The final protein concentration of the purified fraction was less than 10 µg/g diet. Ten ants per treatment, a water source, harborage and the treated diet were placed in sealed plastic containers and maintained in the dark at 27°C in a humidified incubator. Mortality was scored at day 10. No control mortality was seen.

Activity against various lepidopteran larvae was tested as follows. *Photorhabdus* culture broth or purified fractions were applied directly to the surface (~1.5 $cm^2$) of 0.25 ml of standard artificial diet in 30 µl aliquots following dilution in control medium or 10 mM sodium phosphate buffer, pH 7.0, respectively. The diet plates were allowed to air-dry in a sterile flow-hood and the wells were infested with single, neonate larva. European corn borer (*Ostrinia nubilalis*) and corn earworm (*Helicoverpa zea*) eggs were supplied from commercial sources and hatched in-house, whereas beet armyworm (*Spodoptera exigua*), cabbage looper (*Trichoplusia ni*), tobacco budworm (*Heliothis virescens*), codling moth (*Laspeyresia pomonella*) and black cutworm (*Agrotis ipsilon*) larvae were supplied internally. Following infestation with larvae, the diet plates were sealed, placed in a humidified growth chamber and maintained in the dark at 27°C for the appropriate period. Mortality and weight determinations were scored at days 5-7 for *Photorhabdus* culture broth and days 4-7 for the purified fraction. Generally, 16 insects per treatment were used in all studies. Control mortality ranged from 4-12.5% for control medium and was less than 10% for phosphate buffer.

Table 3

Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth and Purified Toxin Fraction on Mortality and Growth Inhibition of Different Insect Orders/Species

| Insect Order/Species | Broth | | Purified Fraction | |
|---|---|---|---|---|
| | % Mort. | % G.I. | % Mort. | % G.I. |
| **COLEOPTERA** | | | | |
| Corn Rootworm | | | | |
| Southern/neonate larva | 100 | na | 100 | na |
| Southern/2nd instar | na | 38.5 | nt | nt |
| Southern/adult | 45 | nt | nt | nt |
| Western/2nd instar | na | 35 | nt | nt |
| Colorado Potato | | | | |
| Beetle | 93 | nt | nt | nt |
| 2nd instar | | | | |
| Turf Grub | na | a.f. | nt | nt |
| 3rd instar | na | a.f. | nt | nt |
| adult | | | | |
| **DIPTERA** | | | | |
| Fruit Fly (adult | 17 | nt | nt | nt |
| emergence) | 100 | na | nt | nt |
| Mosquito larvae | | | | |
| **HOMOPTERA** | | | | |
| Aster Leafhopper | 96.5 | na | 100 | na |
| **HYMENOPTERA** | | | | |
| Argentine Ant | 75 | na | nt | na |
| Carpenter Ant | 71 | na | 100 | na |
| **LEPIDOPTERA** | | | | |
| Beet Armyworm | 12.5 | 36 | 18.75 | 41.4 |
| Black Cutworm | nt | nt | 0 | 71.2 |
| Cabbage Looper | nt | nt | 21.9 | 66.8 |
| Codling Moth | nt | nt | 6.25 | 45.9 |
| Corn Earworm | 56.3 | 94.2 | 97.9 | na |
| European Corn Borer | 96.7 | 98.4 | 100 | na |
| Tobacco Budworm | 13.5 | 52.5 | 19.4 | 85.6 |

Mort. = mortality, G.I. = growth inhibition,
na = not applicable, nt = not tested, a.f. = anti-feedant

Example 3

Insecticide Utility Upon Soil Application

*Photorhabdus luminescens* (strain W-14) culture broth was shown to be active against corn rootworm when applied directly to soil or a soil-mix (Metromix®). Activity against neonate SCR and WCR in Metromix® was tested as follows (Table 4). The test was run using corn seedlings (United Agriseeds brand CL614) that were germinated in the light on moist filter paper for 6 days. After roots were approximately 3-6 cm long, a single kernel/seedling was planted in a 591 ml clear plastic cup with 50 gm of dry Metromix®. Twenty neonate SCR or WCR were then placed directly on the roots of the seedling and covered with Metromix®. Upon infestation, the seedlings were then drenched with 50 ml total volume of a diluted broth solution. After drenching, the cups were sealed and left at room temperature in the light for 7 days. Afterwards, the seedlings were washed to remove all Metromix® and the roots were excised and weighed. Activity was rated as the percentage of corn root remaining relative to the control plants and as leaf damage induced by feeding. Leaf damage was scored visually and rated as either -, +, ++, or +++, with - representing no damage and +++ representing severe damage.

Activity against neonate SCR in soil was tested as follows (Table 5). The test was run using corn seedlings (United Agriseeds brand CL614) that were germinated in the light on moist filter paper for 6 days. After the roots were approximately 3-6 cm long, a single kernel/seedling was planted in a 591 ml clear plastic cup with 150 gm of soil from a field in Lebanon, IN planted the previous year with corn. This soil had not been previously treated with insecticides. Twenty neonate SCR were then placed directly on the roots of the seedling and covered with soil. After infestation, the seedlings were drenched with 50 ml total volume of a diluted broth solution. After drenching, the unsealed cups were incubated in a high relative humidity chamber (80%) at 78°F. Afterwards, the seedlings were washed to remove all soil and the roots were excised and weighed. Activity was rated as the percentage of corn root remaining relative to the control plants and as leaf damage induced by feeding. Leaf damage was scored visually and rated as either -, +, ++, or +++, with - representing no damage and +++ representing severe damage.

Table 4
Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on Rootworm Larvae After Post-Infestation Drenching (Metromix®)

| Treatment | Larvae | Leaf Damage | Root Weight (g) | % |
|---|---|---|---|---|
| **Southern Corn Rootworm** | | | | |
| Water | – | – | 0.4916 ± 0.023 | 100 |
| Medium (2.0% v/v) | – | – | 0.4416 ± 0.029 | 100 |
| Broth (6.25%v/v) | – | – | 0.4641 ± 0.081 | 100 |
| Water | + | +++ | 0.1410 ± 0.006 | 28.7 |
| Media (2.0% v/v) | + | +++ | 0.1345 ± 0.028 | 30.4 |
| Broth (1.56% v/v) | + | – | 0.4830 ± 0.031 | 104 |
| **Western Corn Rootworm** | | | | |
| Water | – | – | 0.4446 ± 0.019 | 100 |
| Broth (2.0% v/v) | – | – | 0.4069 ± 0.026 | 100 |
| Water | + | – | 0.2202 ± 0.015 | 49 |
| Broth (2.0% v/v) | + | – | 0.3879 ± 0.013 | 95 |

Table 5
Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on Southern Corn Rootworm Larvae After Post-Infestation Drenching (Soil)

| Treatment | Larvae | Leaf Damage | Root Weight(g) | % |
|---|---|---|---|---|
| Water | – | – | 0.2148 ± 0.014 | 100 |
| Broth (50% v/v) | – | – | 0.2260 ± 0.016 | 103 |
| Water | + | +++ | 0.0916 ± 0.009 | 43 |
| Broth (50% v/v) | + | – | 0.2428 ± 0.032 | 113 |

Activity of *Photorhabdus luminescens* (strain W-14) culture broth against second instar turf grubs in Metromix® was observed in tests conducted as follows (Table 6). Approximately 50 gm of dry Metromix® was added to a 591 ml clear plastic cup. The Metromix® was then drenched with 50 ml total volume of a 50% (v/v) diluted *Photorhabdus* broth solution. The dilution of crude broth was made with water, with 50% broth being prepared by adding 25 ml of crude broth to 25 ml of water for 50 ml total volume. A 1% (w/v) solution of proteose peptone #3 (PP3), which is a 50% dilution of the normal media concentration, was used as a broth control. After drenching, five second instar turf grubs were

placed on the top of the moistened Metromix®. Healthy turf grub larvae burrowed rapidly into the Metromix®. Those larvae that did not burrow within 1h were removed and replaced with fresh larvae. The cups were sealed and placed in a 28°C incubator, in the dark. After seven days, larvae were removed from the Metromix® and scored for mortality. Activity was rated the percentage of mortality relative to control.

Table 6
Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on Turf Grub After Pre-Infestation Drenching (Metromix®)

| Treatment | Mortality* | Mortality % |
|---|---|---|
| Water | 7/15 | 47 |
| Control medium (1.0% w/v) | 12/19 | 63 |
| Broth (50% v/v) | 17/20 | 85 |

*expressed as a ratio of dead/living larvae

## Example 4
## Insecticide Utility Upon Leaf Application

Activity of *Photorhabdus* broth against European corn borer was seen when the broth was applied directly to the surface of maize leaves (Table 7). In these assays *Photorhabdus* broth was diluted 100-fold with culture medium and applied manually to the surface of excised maize leaves at a rate of ~6.0 µl/cm$^2$ of leaf surface. The leaves were air dried and cut into equal sized strips approximately 2 x 2 inches. The leaves were rolled, secured with paper clips and placed in 1 oz plastic shot glasses with 0.25 inch of 2% agar on the bottom surface to provide moisture. Twelve neonate European corn borers were then placed onto the rolled leaf and the cup was sealed. After incubation for 5 days at 27°C in the dark, the samples were scored for feeding damage and recovered larvae.

Table 7
Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on European Corn Borer Larvae Following Pre-Infestation Application to Excised Maize Leaves

| Treatment | Leaf Damage | Larvae Recovered | Weight(mg) |
|---|---|---|---|
| Water | Extensive | 55/120 | 0.42 mg |
| Control Medium | Extensive | 40/120 | 0.50 mg |
| Broth (1.0% v/v) | Trace | 3/120 | 0.15 mg |

Activity of the culture broth against neonate tobacco budworm (*Heliothis virescens*) was demonstrated using a leaf dip methodology. Fresh cotton leaves were excised from the plant and leaf disks were cut with an 18.5 mm cork-borer. The disks were individually emersed in control medium (PP3) or *Photorhabdus luminescens* (strain W-14) culture broth which had been concentrated approximately 10-fold using an Amicon (Beverly, MA), Proflux M12 tangential filtration system with a 10 kDa filter. Excess liquid was removed and a straightened paper clip was placed through the center of the disk. The paper clip was then wedged into a plastic, 1.0 oz shot glass containing approximately 2.0 ml of 1% Agar. This served to suspend the leaf disk above the agar. Following drying of the leaf disk, a single neonate tobacco budworm larva was placed on the disk and the cup was capped. The cups were then sealed in a plastic bag and placed in a darkened, 27°C incubator for 5 days. At this time the remaining larvae and leaf material were weighed to establish a measure of leaf damage (Table 8).

Table 8
Effect of *Photorhabdus luminescens* (Strain W-14) Culture Broth on Tobacco Budworm Neonates in a Cotton-Leaf Dip Assay

| Treatment | Final Weights (mg) Leaf Disk | Larvae |
|---|---|---|
| Control leaves | 55.7 ± 1.3 | na* |
| Control Medium | 34.0 ± 2.9 | 4.3 ± 0.91 |
| *Photorhabdus* broth | 54.3 ± 1.4 | 0.0** |

* - not applicable, ** - no live larvae found

Example 5, Part A

Characterization of Toxin Peptide Components

In a subsequent analysis, the toxin protein subunits of the bands isolated as in Example 1 were resolved on a 7% SDS polyacrylamide electrophoresis gel with a ratio of 30:0.8 (acrylamide:BIS-acrylamide). This gel matrix facilitates better resolution of the larger proteins. The gel system used to estimate the Band 1 and Band 2 subunit molecular weights in Example 1 was an 18% gel with a ratio of 38:0.18 (acrylamide:BIS-acrylamide), which allowed for a broader range of size separation, but less resolution of higher molecular weight components.

In this analysis, 10, rather than 8, protein bands were resolved. Table 9 reports the calculated molecular weights of the 10 resolved bands, and directly compares the molecular weights estimated under these conditions to those of the prior example. It is not surprising that additional bands were detected under the different separation conditions used in this example. Variations between the prior and new estimates of molecular weight are also to be expected given the differences in analytical conditions. In the analysis of this example, it is thought that the higher molecular weight estimates are more accurate than in Example 1, as a result of improved resolution. However, these are estimates based on SDS PAGE analysis, which are typically not analytically precise and result in estimates of peptides and which may have been further altered due to post- and co-translational modifications.

Amino acid sequences were determined for the N-terminal portions of five of the 10 resolved peptides. Table 9 correlates the molecular weight of the proteins and the identified sequences. In SEQ ID NO:2, certain analyses suggest that the proline at residue 5 may be an asparagine (asn). In SEQ ID NO:3, certain analyses suggest that the amino acid residues at positions 13 and 14 are both arginine (arg). In SEQ ID NO:4, certain analyses suggest that the amino acid residue at position 6 may be either alanine (ala) or serine (ser). In SEQ ID NO:5, certain analyses suggest that the amino acid residue at position 3 may be aspartic acid (asp).

Table 9

| EXAMPLE 1 ESTIMATE | NEW ESTIMATE* | SEQ. LISTING |
|---|---|---|
| 208 | 200.2 kDa | SEQ ID NO:1 |
| 184 | 175.0 kDa | SEQ ID NO:2 |
| 65.6 | 68.1 kDa | SEQ ID NO:3 |
| 60.8 | 65.1 kDa | SEQ ID NO:4 |
| 56.2 | 58.3 kDa | SEQ ID NO:5 |
| 25.1 | 23.2 kDa | SEQ ID NO:15 |

*New estimates are based on SDS PAGE and are not based on gene sequences. SDS PAGE is not analytically precise.

## Example 5, Part B
## Characterization of Toxin Peptide Components

New N-terminal sequence, SEQ ID NO:15, Ala Gln Asp Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr, was obtained by further N-terminal sequencing of peptides isolated from Native HPLC-purified toxin as described in Example 5, Part A, above. This peptide comes from the *tcaA* gene. The peptide labeled $TcaA_{ii}$, starts at position 254 and goes to position 491, where the $TcaA_{iii}$ peptide starts, SEQ ID NO:4. The estimated size of the peptide based on the gene sequence is 25,240 Da.

## Example 6
## Characterization of Toxin Peptide Components

In yet another analysis, the toxin protein complex was re-isolated from the *Photorhabdus luminescens* growth medium (after culture without Tween) by performing a 10% - 80% ammonium sulfate precipitation followed by an ion exchange chromatography step (Mono Q) and two molecular sizing chromatography steps. These conditions were like those used in Example 1. During the first molecular sizing step, a second biologically active peak was found at about 100 ± 10 kDa. Based upon protein measurements, this fraction was 20 - 50 fold less active than the larger, or primary, active peak of about 860 ± 100 kDa (native). During this isolation experiment, a smaller active peak of about 325 ± 50 kDa that retained a considerable portion of the starting biological activity was also resolved. It is thought that the 325 kDa peak is related to or derived from the 860 kDa peak.

A 56 kD protein was resolved in this analysis. The N-terminal sequence of this protein is presented in SEQ ID NO:6. It is noteworthy that this protein shares significant identity and conservation with SEQ ID NO:5 at the N-terminus, suggesting that the two may be encoded by separate members of a gene family and that the proteins produced by each gene are sufficiently similar to both be operable in the insecticidal toxin complex.

A second, prominent 185 kDa protein was consistently present in amounts comparable to that of protein 3 from Table 9, and may be the same protein or protein fragment. The N-terminal sequence of this 185 kDa protein is shown at SEQ ID NO:7.

Additional N-terminal amino acid sequence data were also obtained from isolated proteins. None of the determined N-terminal sequences appear identical to a protein identified in Table 9. Other proteins were present in isolated preparation. One such protein has an estimated molecular weight of 108 kDa and an N-terminal sequence as shown in SEQ ID NO:8. A second such protein has an estimated molecular weight of 80 kDa and an N-terminal sequence as shown in SEQ ID NO:9.

When the protein material in the approximately 325 kDa active peak was analyzed by size, bands of approximately 51, 31, 28, and 22 kDa were observed. As in all cases in which a molecular weight was determined by analysis of electrophoretic mobility, these molecular weights were subject to error effects introduced by buffer ionic strength differences, electrophoresis power differences, and the like. One of ordinary skill would understand that definitive molecular weight values cannot be determined using these standard methods and that each was subject to variation. It was hypothesized that proteins of these sizes are degradation products of the larger protein species (of approximately 200 kDa size) that were observed in the larger primary toxin complex.

Finally, several preparations included a protein having the N-terminal sequence shown in SEQ ID NO:10. This sequence was strongly homologous to known chaperonin proteins, accessory proteins known to function in the assembly of large protein complexes. Although the applicants could not ascribe such an assembly function to the protein identified in SEQ ID NO:10, it was consistent with the existence of the described toxin protein complex that such a chaperonin protein could be involved in its

assembly. Moreover, although such proteins have not directly been suggested to have toxic activity, this protein may be important to determining the overall structural nature of the protein toxin, and thus, may contribute to the toxic activity or durability of the complex *in vivo* after oral delivery.

Subsequent analysis of the stability of the protein toxin complex to proteinase K was undertaken. It was determined that after 24 hour incubation of the complex in the presence of a 10-fold molar excess of proteinase K, activity was virtually eliminated (mortality on oral application dropped to about 5%). These data confirm the proteinaceous nature of the toxin.

The toxic activity was also retained by a dialysis membrane, again confirming the large size of the native toxin complex.

## Example 7

## Isolation, Characterization and Partial Amino Acid Sequencing of *Photorhabdus* Toxins

Isolation and N-Terminal Amino Acid Sequencing: In a set of experiments conducted in parallel to Examples 5 and 6, ammonium sulfate precipitation of *Photorhabdus* proteins was performed by adjusting *Photorhabdus* broth, typically 2-3 liters, to a final concentration of either 10% or 20% by the slow addition of ammonium sulfate crystals. After stirring for 1 hour at 4°C, the material was centrifuged at 12,000 x g for 30 minutes. The supernatant was adjusted to 80% ammonium sulfate, stirred at 4°C for 1 hour, and centrifuged at 12,000 x g for 60 minutes. The pellet was resuspended in one-tenth the volume of 10 mM $Na_2 \cdot PO_4$, pH 7.0 and dialyzed against the same phosphate buffer overnight at 4°C. The dialyzed material was centrifuged at 12,000 x g for 1 hour prior to ion exchange chromatography.

A HR 16/50 Q Sepharose (Pharmacia) anion exchange column was equilibrated with 10 mM $Na_2 \cdot PO_4$, pH 7.0. Centrifuged, dialyzed ammonium sulfate pellet was applied to the Q Sepharose column at a rate of 1.5 ml/min and washed extensively at 3.0 ml/min with equilibration buffer until the optical density (O.D. 280) reached less than 0.100. Next, either a 60 minute NaCl gradient ranging from 0 to 0.5 M at 3 ml/min, or a series of step elutions using 0.1 M, 0.4 M and finally 1.0 NaCl for 60 minutes each was applied to the column. Fractions were pooled and concentrated using a

Centriprep 10[redacted] Alternatively, proteins co[redacted]d be eluted by a single 0.4 M NaCl wash without prior elution with 0.1 M NaCl.

Two milliliter aliquots of concentrated Q Sepharose samples were loaded at 0.5 ml/min onto a HR 16/50 Superose 12 (Pharmacia) gel filtration column equilibrated with 10 mM $Na_2 \cdot PO_4$, pH 7.0. The column was washed with the same buffer for 240 min at 0.5 ml/min and 2 min samples were collected. The void volume material was collected and concentrated using a Centriprep 100. Two milliliter aliquots of concentrated Superose 12 samples were loaded at 0.5 ml/min onto a HR 16/50 Sepharose 4B-CL (Pharmacia) gel filtration column equilibrated with 10 mM $Na_2 \cdot PO_4$, pH 7.0. The column was washed with the same buffer for 240 min at 0.5 ml/min and 2 min samples were collected.

The excluded protein peak was subjected to a second fractionation by application to a gel filtration column that used a Sepharose CL-4B resin, which separates proteins ranging from ~30 kDa to 1000 kDa. This fraction was resolved into two peaks; a minor peak at the void volume (>1000 kDa) and a major peak which eluted at an apparent molecular weight of about 860 kDa. Over a one week period subsequent samples subjected to gel filtration showed the gradual appearance of a third peak (approximately 325 kDa) that seemed to arise from the major peak, perhaps by limited proteolysis. Bioassays performed on the three peaks showed that the void peak had no activity, while the 860 kDa toxin complex fraction was highly active, and the 325 kDa peak was less active, although quite potent. SDS PAGE analysis of Sepharose CL-4B toxin complex peaks from different fermentation productions revealed two distinct peptide patterns, denoted "P" and "S". The two patterns had marked differences in the molecular weights and concentrations of peptide components in their fractions. The "S" pattern, produced most frequently, had 4 high molecular weight peptides (> 150 kDa) while the "P" pattern had 3 high molecular weight peptides. In addition, the "S" peptide fraction was found to have 2-3 fold more activity against European Corn Borer. This shift may be related to variations in protein expression due to age of inoculum and/or other factors based on growth parameters of aged cultures.

Milligram quantities of peak toxin complex fractions determined to be "P" or "S" peptide patterns were subjected to preparative SDS PAGE, and transblotted with TRIS-glycine

(Seprabuff™ to PVDF membranes (ProBlott™, Applied Biosystems) for 3-4 hours. Blots wer sent for amino acid analysis and N-terminal amino acid sequencing at Harvard MicroChem and Cambridge ProChem, respectively. Three peptides in the "S" pattern had unique N-terminal amino acid sequences compared to the sequences identified in the previous example. A 201 kDa ($TcdA_{ii}$) peptide set forth as SEQ ID NO:.13 below shared between 33% amino acid identity and 50% similarity with SEQ ID NO:1 ($TcbA_{ii}$)(Table 10, in Table 10 vertical lines denote amino acid identities and colons indicate conservative amino acid substitutions). A second peptide of 197 kDa, SEQ ID NO:14 (TcdB), had 42% identity and 58% homology with SEQ ID NO:2 (TcaC). Yet a third peptide of 205 kDa was denoted $TcdA_{ii}$. In addition, a limited N-terminal amino acid sequence, SEQ ID NO:16 (TcbA), of a peptide of at least 235 kDa was identical in homology with the amino acid sequence, SEQ ID NO:12, deduced from a cloned gene (*tcbA*), SEQ ID NO:11, containing a deduced amino acid sequence corresponding to SEQ ID NO:1 ($TcbA_{ii}$). This indicates that the larger 235+ kDa peptide was proteolytically processed to the 201 kDa peptide, ($TcbA_{ii}$), (SEQ ID NO:1) during fermentation, possibly resulting in activation of the molecule. In yet another sequence, the sequence originally reported as SEQ ID NO:5 ($TcaB_{ii}$) reported in Example 5 above, was found to contain an aspartic acid residue (Asp) at the third position rather than glycine (Gly) and two additional amino acids Gly and Asp at the eighth and ninth positions, respectively. In yet two other sequences, SEQ ID NO:2 (TcaC) and SEQ ID NO:3 ($TcaB_i$), additional amino acid sequence was obtained. Densitometric quantitation was performed using a sample that was identical to the "S" preparation sent for N-terminal analysis. This analysis showed that the 201 kDa and 197 kDa peptides represent 7.0% and 7.2%, respectively, of the total Coomassie brillant blue stained protein in the "S" pattern and are present in amounts similar to the other abundant peptides. It is speculated that these peptides may represent protein homologs, analogous to the situation found with other bacterial toxins, such as various CryI Bt toxins. These proteins vary from 40-90% homology at their N-terminal amino acid sequence, which encompasses the toxic fragment.

Intern[redacted]Amino Acid Sequencing: To litate cloning of toxin peptide genes, internal amino acid sequences of selected peptides were obtained as followed. Milligram quantities of peak 2A fractions determined to be "P" or "S" peptide patterns were subjected to preparative SDS PAGE, and transblotted with TRIS-glycine (Seprabuff™ to PVDF membranes (ProBlott™, Applied Biosystems) for 3-4 hours. Blots were sent for amino acid analysis and N-terminal amino acid sequencing at Harvard MicroChem and Cambridge ProChem, respectively. Three peptides, referred to as $TcbA_{ii}$ (containing SEQ ID NO:1), $TcdA_{ii}$, and $TcaB_i$ (containing SEQ ID NO:3) were subjected to trypsin digestion by Harvard MicroChem followed by HPLC chromatography to separate individual peptides. N-terminal amino acid analysis was performed on selected tryptic peptide fragments. Two internal peptides were sequenced for the peptide $TcaB_i$ (205 kDa peptide) referred to as $TcaB_i$-PT111 (SEQ ID NO:17) and $TcaB_i$-PT79 (SEQ ID NO:18). Two internal peptides were sequenced for the peptide $TcaB_i$ (68 kDa peptide) referred to as $TcaB_i$-PT158 (SEQ ID NO:19) and $TcaB_i$-PT108 (SEQ ID NO:20). Four internal peptides were sequenced for the peptide $TcbA_{ii}$ (201 kDa peptide) referred to as TCBAII-PT103 (SEQ ID NO:21), $TcbA_{ii}$-PT56 (SEQ ID NO:22), $TcbA_{ii}$-PT81(a) (SEQ ID NO:23), and $TcbA_{ii}$-PT81(b) (SEQ ID NO:24).

Table 10

N-Terminal Amino Acid Sequences

```
201 kDa (33% identity & 50% similarity to SEQ ID NO.1)
  L I G Y N N Q F S G * A     SEQ ID NO:13
  :   | |       | :     |
F I Q G Y S D L F G N - A     SEQ ID NO:1

197 kDa (42% identity & 58% similarity SEQ ID NO.2)
M Q N S Q T F S V G E L       SEQ ID NO.14
| | : |       | : :   |
M Q D S P E V S I T T L       SEQ ID NO.2
```

## Example 8

## Construction of a cosmid library of *Photorhabdus luminescens* W-14 genomic DNA and its screening to isolate genes encoding peptides comprising the toxic protein preparation

As a prerequisite for the production of *Photorhabdus* insect toxic proteins in heterologous hosts, and for other uses, it is n cessary to isolate and characterize the genes that encode those

peptides. This objective was pursued in parallel. One approach, described later, was based on the use of monoclonal and polyclonal antibodies raised against the purified toxin which were then used to isolate clones from an expression library. The other approach, described in this example, is based on the use of the N-terminal and internal amino acid sequence data to design degenerate oligonucleotides for use in PCR amplication. Either method can be used to identify DNA clones that contain the peptide-encoding genes so as to permit the isolation of the respective genes, and the determination of their DNA base sequence.

GENOMIC DNA ISOLATION: *Photorhabdus luminescens* strain W-14 (ATCC accession number 55397) was grown on 2% proteose peptone #3 agar (Difco Laboratories, Detroit, MI) and insecticidal toxin competence was maintained by repeated bioassay after passage, using the method described in Example 1 above. A 50 ml shake culture was produced in a 175 ml baffled flask in 2% proteose peptone #3 medium, grown at 28°C and 150 rpm for approximately 24 hours. 15 ml of this culture was pelleted and frozen in its medium at -20°C until it was thawed for DNA isolation. The thawed culture was centrifuged, (700 x g, 30 min) and the floating orange mucopolysaccharide material was removed. The remaining cell material was centrifuged (25,000 x g, 15 min) to pellet the bacterial cells, and the medium was removed and discarded.

Genomic DNA was isolated by an adaptation of the CTAB method described in section 2.4.1 of Current Protocols in Molecular Biology (Ausubel *et al.* eds, John Wiley & Sons, 1994) [modified to include a salt shock and with all volumes increased 10-fold]. The pelleted bacterial cells were resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a final volume of 10 ml, then 12 ml of 5 M NaCl was added; this mixture was centrifuged 20 min at 15,000 x g. The pellet was resuspended in 5.7 ml TE and 300 ml of 10% SDS and 60 ml of 20 mg/ml proteinase K (Gibco BRL Products, Grand Island, NY; in sterile distilled water) were added to the suspension. This mixture was incubated at 37°C for 1 hr; then approximately 10 mg lysozyme (Worthington Biochemical Corp., Freehold, NJ) was added. After an additional 45 min, 1 ml of 5 M NaCl and 800 ml of CTAB/NaCl solution (10% w/v CTAB, 0.7 M

NaCl) were added. This preparation was incubated 10 min at 65°C, then gently agitated and further incubated and agitated for approximately 20 min to assist clearing of the cellular material. An equal volume of chloroform/isoamyl alcohol solution (24:1, v/v) was added, mixed gently and centrifuged. After two extractions with an equal volume of PCI (phenol/chloroform/isoamyl alcohol; 50:49:1, v/v/v; equilibrated with 1 M Tris-HCl, pH 8.0; Intermountain Scientific Corporation, Kaysville, UT), the DNA was precipitated with 0.6 volume of isopropanol. The DNA precipitate was gently removed with a glass rod, washed twice with 70% ethanol, dried, and dissolved in 2 ml STE (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 1 mM EDTA). This preparation contained 2.5 mg/ml DNA, as determined by optical density at 260 nm (i.e., $OD_{260}$).

The molecular size range of the isolated genomic DNA was evaluated for suitability for library construction. CHEF gel analysis was performed in 1.5% agarose (Seakem® LE, FMC BioProducts, Rockland, ME) gels with 0.5 X TBE buffer (44.5 mM Tris-HCl pH 8.0, 44.5 mM $H_3BO_3$, 1 mM EDTA) on a BioRad CHEF-DR II apparatus with a Pulsewave 760 Switcher (Bio-Rad Laboratories, Inc., Richmond, CA). The running parameters were: initial A time, 3 sec; final A time, 12 sec; 200 volts; running temperature, 4-18°C; run time, 16.5 hr. Ethidium bromide staining and examination of the gel under ultraviolet light indicated the DNA ranged from 30-250 kbp in size.

CONSTRUCTION OF LIBRARY: A partial Sau3A 1 digest was made of this *Photorhabdus* genomic DNA preparation. The method was based on section 3.1.3 of Ausubel (*supra.*). Adaptions included running smaller scale reactions under various conditions until nearly optimal results were achieved. Several scaled-up large reactions with varied conditions were run, the results analyzed on CHEF gels, and only the best large scale preparation was carried forward. In the optimal case, 200 µg of *Photorhabdus* genomic DNA was incubated with 1.5 units of Sau3A 1 (New England Biolabs, "NEB", Beverly, MA) for 15 min at 37°C in 2 ml total volume of 1X NEB 4 buffer (supplied as 10X by the manufacturer). The reaction was stopped by adding 2 ml of PCI and centrifuging at 8000 x g for 10 min. To the supernatant were added 200 µl of 5 M NaCl plus 6 ml of ice-cold ethanol. This preparation was

chilled 30 min at -20°C, then centrifuged at 12,000 x g for 15 min. The supernatant was removed and the precipitate was dried in a vacuum oven at 40°C, then resuspended in 400 µl STE. Spectrophotometric assay indicated about 40% recovery of the input DNA. The digested DNA was size fractionated on a sucrose gradient according to section 5.3.2 of CPMB (*op. cit.*). A 10% to 40% (w/v) linear sucrose gradient was prepared with a gradient maker in Ultra-Clear™ tubes (Beckman Instruments, Inc., Palo Alto, CA) and the DNA sample was layered on top. After centrifugation, (26,000 rpm, 17 hr, Beckman SW41 rotor, 20°C), fractions (about 750 µl) were drawn from the top of the gradient and analyzed by CHEF gel electrophoresis (as described earlier). Fractions containing Sau3A 1 fragments in the size range 20-40 kbp were selected and DNA was precipitated by a modification (amounts of all solutions increased approximately 6.3-fold) of the method in section 5.3.3 of Ausubel (*supra.*). After overnight precipitation, the DNA was collected by centrifugation (17,000 x g, 15 min), dried, redissolved in TE, pooled into a final volume of 80 µl, and reprecipitated with the addition of 8 µl 3 M sodium acetate and 220 µl ethanol. The pellet collected by centrifugation as above was resuspended in 12 µl TE. Concentration of the DNA was determined by Hoechst 33258 dye (Polysciences, Inc., Warrington, PA) fluorometry in a Hoefer TKO100 fluorimeter (Hoefer Scientific Instruments, San Francisco, CA). Approximately 2.5 µg of the size-fractionated DNA was recovered.

Thirty µg of cosmid pWE15 DNA (Stratagene, La Jolla, CA) was digested to completion with 100 units of restriction enzyme BamH 1 (NEB) in the manufacturer's buffer (final volume of 200 µl, 37°C, 1 hr). The reaction was extracted with 100 µl of PCI and DNA was precipitated from the aqueous phase by addition of 20 µl 3M sodium acetate and 550 µl -20°C absolute ethanol. After 30 min at -70°C, the DNA was collected by centrifugation (17,000 x g, 15 min), dried under vacuum, and dissolved in 180 µl of 10 mM Tris-HCl, pH 8.0. To this were added 20 µl of 10X CIP buffer (100 mM Tris-HCl, pH 8.3; 10 mM $ZnCl_2$; 10 mM $MgCl_2$), and 1 µl (0.25 units) of 1:4 diluted calf intestinal alkaline phosphatase

(Boehringer [redacted]heim Corporation, Indianapo[redacted], IN). After 30 min at 37°C, the following additions were made: 2 µl 0.5 M EDTA, pH 8.0; 10 µl 10% SDS; 0.5 µl of 20 mg/ml proteinase K (as above), followed by incubation at 55°C for 30 min. Following sequential extractions with 100 µl of PCI and 100 µl phenol (Intermountain Scientific Corporation, equilibrated with 1 M Tris-HCl, pH 8.0), the dephosphorylated DNA was precipitated by addition of 72 µl of 7.5 M ammonium acetate and 550 µl -20°C ethanol, incubation on ice for 30 min, and centrifugation as above. The pelleted DNA was washed once with 500 µl -20°C 70% ethanol, dried under vacuum, and dissolved in 20 µl of TE buffer.

Ligation of the size-fractionated Sau3A 1 fragments to the BamH 1-digested and phosphatased pWE15 vector was accomplished using T4 ligase (NEB) by a modification (i.e., use of premixed 10X ligation buffer supplied by the manufacturer) of the protocol in section 3.33 of Ausubel. Ligation was carried out overnight in a total volume of 20 µl at 15°C, followed by storage at -20°C.

Four µl of the cosmid DNA ligation reaction, containing about 1 µg of DNA, was packaged into bacteriophage lambda using a commercial packaging extract (Gigapack® III Gold Packaging Extract, Stratagene), following the manufacturer's directions. The packaged preparation was stored at 4°C until use. The packaged cosmid preparation was used to infect *Escherichia coli* XL1 Blue MR cells (Stratagene) according to the Gigapack® III Gold protocols ("Titering the Cosmid Library"), as follows. XL1 Blue MR cells were grown in LB medium (g/L: Bacto-tryptone, 10; Bacto-yeast extract, 5; Bacto-agar, 15; NaCl, 5; [Difco Laboratories, Detroit, MI]) containing 0.2% (w/v) maltose plus 10 mM $MgSO_4$, at 37°C. After 5 hr growth, cells were pelleted at 700 x g (15 min) and resuspended in 6 ml of 10 mM $MgSO_4$. The culture density was adjusted with 10 mM $MgSO_4$ to $OD_{600}$ = 0.5. The packaged cosmid library was diluted 1:10 or 1:20 with sterile SM medium (0.1 M NaCl, 10 mM $MgSO_4$, 50 mM Tris-HCl pH 7.5, 0.01% w/v gelatin), and 25 µl of the diluted preparation was mixed with 25 µl of the diluted XL1 Blue MR cells. The mixture was incubated at 25°C for 30 min (without shaking), then 200 µl of LB broth was added, and incubation was continued for approximately 1 hr with occasional

gentle shaking. Aliquots (20-40 µl) of this culture were spread on LB agar plates containing 100 mg/l ampicillin (i.e., LB-$Amp_{100}$) and incubated overnight at 37°C. To store the library without amplification, single colonies were picked and inoculated into individual wells of sterile 96-well microwell plates; each well containing 75 µl of Terrific Broth (TB media: 12 g/l Bacto-tryptone, 24 g/l Bacto-yeast extract, 0.4% v/v glycerol, 17 mM $KH_2PO_4$, 72 mM $K_2HPO_4$) plus 100 mg/l ampicillin (i.e., TB-$Amp_{100}$) and incubated (without shaking) overnight at 37°C. After replicating the 96-well plate into a copy plate, 75 µl/well of filter-sterilized TB:glycerol (1:1, v/v; with, or without, 100 mg/l ampicillin) was added to the plate, it was shaken briefly at 100 rpm, 37°C, and then closed with Parafilm® (American National Can, Greenwich, CT) and placed in a -70°C freezer for storage. Copy plates were grown and processed identically to the master plates. A total of 40 such master plates (and their copies) were prepared.

SCREENING OF THE LIBRARY WITH RADIOLABELED DNA PROBES: To prepare colony filters for probing with radioactively labeled probes, ten 96-well plates of the library were thawed at 25°C (bench top at room temperature). A replica plating tool with 96 prongs was used to inoculate a fresh 96-well copy plate containing 75 µl/well of TB-$Amp_{100}$. The copy plate was grown overnight (stationary) at 37°C, then shaken about 30 min at 100 rpm at 37°C. A total of 800 colonies was represented in these copy plates, due to nongrowth of some isolates. The replica tool was used to inoculate duplicate impressions of the 96-well arrays onto Magna NT (MSI, Westboro, MA) nylon membranes (0.45 micron, 220 x 250 mm) which had been placed on solid LB-$Amp_{100}$ (100 ml/dish) in Bio-assay plastic dishes (Nunc, 243 x 243 x 18 mm; Curtin Mathison Scientific, Inc., Wood Dale, IL). The colonies were grown on the membranes at 37°C for about 3 hr.

A positive control colony (a bacterial clone containing a GZ4 sequence insert, see below) was grown on a separate Magna NT membrane (Nunc, 0.45 micron, 82 mm circle) on LB medium supplemented with 35 mg/l chloramphenicol (i.e., LB-$Cam_{35}$), and processed alongside the library colony membranes. Bacterial colonies on the membranes were lysed, and th DNA was denatured

and neutrali[redacted] according to a protocol ta from the Genius™ System User's Guide version 2.0 (Boehringer Mannheim, Indianapolis, IN). Membranes were placed colony side up on filter paper soaked with 0.5 N NaOH plus 1.5 M NaCl for 15 min to denature, and neutralized on filter paper soaked with 1 M Tris-HCl pH 8.0, 1.5 M NaCl for 15 min. After UV-crosslinking using a Stratagene UV Stratalinker set on auto crosslink, the membranes were stored dry at 25°C until use. Membranes were trimmed into strips containing the duplicate impressions of a single 96-well plate, then washed extensively by the method of section 6.4.1 in CPMB (*op. cit.*): 3 hr at 25°C in 3X SSC, 0.1% (w/v) SDS, followed by 1 hr at 65°C in the same solution, then rinsed in 2X SSC in preparation for the hybridization step (20X SSC = 3 M NaCl, 0.3 M sodium citrate, pH 7.0).

Amplification of a specific genomic fragment of a *tcaC* gene. Based on the N-terminal amino acid sequence determined for the purified TcaC peptide fraction [disclosed herein as SEQ ID NO:2], a pool of degenerate oligonucleotides (pool S4Psh) was synthesized by standard β-cyanoethyl chemistry on an Applied BioSystem ABI394 DNA/RNA Synthesizer (Perkin Elmer, Foster City, CA). The oligonucleotides were deprotected 8 hours at 55°C, dissolved in water, quantitated by spectrophotometric measurement, and diluted for use. This pool corresponds to the determined N-terminal amino acid sequence of the TcaC peptide. The determined amino acid sequence and the corresponding degenerate DNA sequence are given below, where A, C, G, and T are the standard DNA bases, and I represents inosine:

| Amino Acid | Met | Gln | Asp | Ser | Pro | Glu | Val |
|---|---|---|---|---|---|---|---|
| S4Psh | 5' ATG | CA(A/G) | GA(T/C) | (T/A)(C/G)(T/A) | CCI | GA(A/G) | GT 3' |

Another set of degenerate oligonucleotides was synthesized (pool P2.3.5R), representing the complement of the coding strand for the determined amino acid sequence of the SEQ ID NO:17:

| Amino Acid | Ala | Phe | Asn | Ile | Asp | Asp | Val |
|---|---|---|---|---|---|---|---|
| Codons | 5' GCN | TT(T/C) | AA(T/C) | AT(A/T/C) | GA(T/C) | GA(T/C) | GT 3' |
| P2.3.5R | 3'CG(A/C/G/T) | AA(A/G) | TT(A/G) | TA(T/A/G) | CT(A/G) | CT(A/G) | CA 5' |

These oligonucleotides were used as primers in Polymerase Chain Reactions (PCR®, Roche Molecular Systems, Branchburg, NJ) to

amplify a specific DNA fragment from genomic DNA prepared from *Photorhabdus* strain W-14 (see above). A typical reaction (50 µl) contained 125 pmol of each primer pool P2Psh and P2.3.5R, 253 ng of genomic template DNA, 10 nmol each of dATP, dCTP, dGTP, and dTTP, 1X GeneAmp® PCR buffer, and 2.5 units of AmpliTaq® DNA polymerase (both from Roche Molecular Systems; 10X GeneAmp® buffer is 100 mM Tris-HCl pH 8.3, 500 mM KCl, 0.01% w/v gelatin). Amplifications were performed in a Perkin Elmer Cetus DNA Thermal Cycler (Perkin Elmer, Foster City, CA) using 35 cycles of 94°C (1.0 min), 55°C (2.0 min), 72°C (3.0 min), followed by an extension period of 7.0 min at 72°C. Amplification products were analyzed by electrophoresis through 2% w/v NuSieve® 3:1 agarose (FMC BioProducts) in TEA buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.0). A specific product of estimated size 250 bp was observed amongst numerous other amplification products by ethidium bromide (0.5 µg/ml) staining of the gel and examination under ultraviolet light.

The region of the gel containing an approximately 250 bp product was excised, and a small plug (0.5 mm dia.) was removed and used to supply template for PCR amplification (40 cycles). The reaction (50 µl) contained the same components as above, minus genomic template DNA. Following amplification, the ends of the fragments were made blunt and were phosphorylated by incubation at 25°C for 20 min with 1 unit of T4 DNA polymerase (NEB), 1 nmol ATP, and 2.15 units of T4 kinase (Pharmacia Biotech Inc., Piscataway, NJ).

DNA fragments were separated from residual primers by electrophoresis through 1% w/v GTG® agarose (FMC) in TEA. A gel slice containing fragments of apparent size 250 bp was excised, and the DNA was extracted using a Qiaex kit (Qiagen Inc., Chatsworth, CA).

The extracted DNA fragments were ligated to plasmid vector pBC KS(+) (Stratagene) that had been digested to completion with restriction enzyme Sma 1 and extracted in a manner similar to that described for pWE15 DNA above. A typical ligation reaction (16.3 µl) contained 100 ng of digested pBC KS(+) DNA, 70 ng of 250 bp fragment DNA, 1 nmol $[Co(NH_3)_6]Cl_3$, and 3.9 Weiss units of T4 DNA ligase (Collaborative Biomedical Products, Bedford, MA), in 1X ligation buffer (50 mM Tris-HCl, pH 7.4; 10 mM $MgCl_2$; 10 mM

dithiothreitol; 1 mM spermidine, 1 mM ATP, 100 mg/ml bovine serum albumin). Following overnight incubation at 14°C, the ligated products were transformed into frozen, competent *Escherichia coli* DH5α cells (Gibco BRL) according to the suppliers' recommendations, and plated on LB-Cam$_{35}$ plates, containing IPTG (119 μg/ml) and X-gal (50 μg/ml). Independent white colonies were picked, and plasmid DNA was prepared by a modified alkaline-lysis/PEG precipitation method (PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit Protocols; ABI/Perkin Elmer). The nucleotide sequence of both strands of the insert DNA was determined, using T7 primers [pBC KS(+) bases 601-623: TAAAACGACGGCCAGTGAGCGCG) and LacZ primers [pBC KS(+) bases 792-816: ATGACCATGATTACGCCAAGCGCGC) and protocols supplied with the PRISM™ sequencing kit (ABI/Perkin Elmer). Nonincorporated dye-terminator dideoxyribonucleotides were removed by passage through Centri-Sep 100 columns (Princeton Separations, Inc., Adelphia, NJ) according to the manufacturer's instructions. The DNA sequence was obtained by analysis of the samples on an ABI Model 373A DNA Sequencer (ABI/Perkin Elmer). The DNA sequences of two isolates, GZ4 and HB14, were found to be as illustrated in Figure 1.

This sequence illustrates the following features: i) bases 1-20 represent one of the 64 possible sequences of the S4Psh degenerate oligonucleotides, ii) the sequence of amino acids 1-3 and 6-12 correspond exactly to that determined for the N-terminus of TcaC (disclosed as SEQ ID NO:2), iii) the fourth amino acid encoded is a cysteine residue rather than serine. This difference is encoded within the degeneracy for the serine codons (see above), iv) the fifth amino acid encoded is proline, corresponding to the TcaC N-terminal sequence given as SEQ ID NO:2, v) bases 257-276 encode one of the 192 possible sequences designed into the degenerate pool, vi) the TGA termination codon introduced at bases 268-270 is the result of complementarity to the degeneracy built into the oligonucleotide pool at the corresponding position, and does not indicate a shortened reading frame for the corresponding gene.

Labeling of a TcaC peptide gene-specific probe. DNA fragments corresponding to the above 276 bases were amplified (35

cycles) CR[®] in a 100 µl reaction volume, using 100 pmol each of P2Psh and P2.3.5R primers, 10 ng of plasmids GZ4 or HB14 as templates, 20 nmol each of dATP, dCTP, dGTP, and dTTP, 5 units of AmpliTAq® DNA polymerase, and 1X concentration of GeneAmp® buffer, under the same temperature regimes as described above. The amplification products were extracted from a 1% GTG® agarose gel by Qiaex kit and quantitated by fluorometry.

The extracted amplification products from plasmid HB14 template (approximately 400 ng) were split into five aliquots and labeled with $^{32}$P-dCTP using the High Prime Labeling Mix (Boehringer Mannheim) according to the manufacturer's instructions. Nonincorporated radioisotope was removed by passage through NucTrap® Probe Purification Columns (Stratagene), according to the supplier's instructions. The specific activity of the labeled DNA product was determined by scintillation counting to be $3.11 \times 10^8$ dpm/µg. This labeled DNA was used to probe membranes prepared from 800 members of the genomic library.

Screening with a TcaC-peptide gene specific probe. The radiolabeled HB14 probe was boiled approximately 10 min, then added to "minimal hyb" solution. [Note: The "minimal hyb" method is taken from a CERES protocol; "Restriction Fragment Length Polymorphism Laboratory Manual version 4.0", sections 4-40 and 4-47; CERES/NPI, Salt Lake City, UT. NPI is now defunct, with its successors operating as Linkage Genetics]. "Minimal hyb" solution contains 10% w/v PEG (polyethylene glycol, M.W. approx. 8000), 7% w/v SDS; 0.6X SSC, 10 mM sodium phosphate buffer (from a 1M stock containing 95 g/l $NaH_2PO_4 \cdot 1H_2O$ and 84.5 g/l $Na_2HPO_4 \cdot 7H_2O$), 5 mM EDTA, and 100 mg/ml denatured salmon sperm DNA. Membranes were blotted dry briefly then, without prehybridization, 5 strips of membrane were placed in each of 2 plastic boxes containing 75 ml of "minimal hyb" and 2.6 ng/ml of radiolabeled HB14 probe. These were incubated overnight with slow shaking (50 rpm) at 60°C. The filters were washed three times for approximately 10 min each at 25°C in "minimal hyb wash solution" (0.25X SSC, 0.2% SDS), followed by two 30-min washes with slow shaking at 60°C in the same solution. The filters were placed on paper covered with Saran Wrap® (Dow Brands, Indianapolis, IN) in a light-tight autoradiographic cassette and exposed to X-Omat X-ray film (Kodak, Rochester, NY) with two

DuPont Cron[redacted] Lightning-Plus Cl enhancers [redacted]igma Chemical Co., St. Louis, MO), for 4 hr at -70°C. Upon development (standard photographic procedures), significant signals were evident in both replicates amongst a high background of weaker, more irregular signals. The filters were again washed for about 4 hr at 68°C in "minimal hyb wash solution" and then placed again in the cassettes and film was exposed overnight at -70°C. Twelve possible positives were identified due to strong signals on both of the duplicate 96-well colony impressions. No signal was seen with negative control membranes (colonies of XL1 Blue MR cells containing pWE15), and a very strong signal was seen with positive control membranes (DH5α cells containing the GZ4 isolate of the PCR product) that had been processed concurrently with the experimental samples.

The twelve putative hybridization-positive colonies were retrieved from the frozen 96-well library plates and grown overnight at 37°C on solid LB-$Amp_{100}$ medium. They were then patched (3/plate, plus three negative controls: XL1 Blue MR cells containing the pWE15 vector) onto solid LB-$Amp_{100}$. Two sets of membranes (Magna NT nylon, 0.45 micron) were prepared for hybridization. The first set was prepared by placing a filter directly onto the colonies on a patch plate, then removing it with adherent bacterial cells, and processing as below. Filters of the second set were placed on plates containing LB-$Amp_{100}$ medium, then inoculated by transferring cells from the patch plates onto the filters. After overnight growth at 37°C, the filters were removed from the plates and processed.

Bacterial cells on the filters were lysed and DNA denatured by placing each filter colony-side-up on a pool (1.0 ml) of 0.5 M NaOH in a plastic plate for 3 min. The filters were blotted dry on a paper towel, then the process was repeated with fresh 0.5 N NaOH. After blotting dry, the filters were neutralized by placing each on a 1.0 ml pool of 1 M Tris-HCl, pH 7.5 for 3 min, blotted dry, and reneutralised with fresh buffer. This was followed by two similar soakings (5 min each) on pools of 0.5 M Tris-HCl pH 7.5 plus 1.5 M NaCl. After blotting dry, the DNA was UV crosslinked to the filter (as above), and the filters were washed (25°C, 100 rpm) in about 100 ml of 3X SSC plus 0.1%(w/v) SDS (4 times, 30 min each with fresh solution for each wash). They were then placed in a minimal volume of prehybridization

solution SSC plus 1% w/v each of Ficoll 400 (Pharmacia), polyvinylpyrrolidone (av. M.W. 360,000; Sigma ) and bovine serum albumin Fraction V; (Sigma)] for 2 hr at 65°C, 50 rpm. The prehybridization solution was removed, and replaced with the HB14 $^{32}$P-labeled probe that had been saved from the previous hybridization of the library membranes and which had been denatured at 95°C for 5 min. Hybridization was performed at 60°C for 16 hr with shaking at 50 rpm.

Following removal of the labeled probe solution, the membranes were washed 3 times at 25°C (50 rpm, 15 min) in 3X SSC (about 150 ml each wash). They were then washed for 3 hr at 68°C (50 rpm) in 0.25X SSC plus 0.2% SDS (minimal hyb wash solution), and exposed to X-ray film as described above for 1.5 hr at 25°C (no enhancer screens). This exposure revealed very strong hybridization signals to cosmid isolates 22G12, 25A10, 26A5, and 26B10, and a very weak signal with cosmid isolate 8B10. No signal was seen with the negative control (pWE15) colonies, and a very strong signal was seen with positive control membranes (DH5α cells containing the GZ4 isolate of the PCR product) that had been processed concurrently with the experimental samples.

Amplification of a specific genomic fragment of a *tcaB* gene. Based on the N-terminal amino acid sequence determined for the purified TcaB$_i$ peptide fraction (disclosed here as SEQ ID NO:3) a pool of degenerate oligonucleotides (pool P8F) was synthesized as described for peptide TcaC. The determined amino acid sequence and the corresponding degenerate DNA sequence are given below, where A, C, G, and T are the standard DNA bases, and I represents inosine:

| Amino Acid | Leu | Phe | Thr | Gln | Thr | Leu | Lys | Glu | Ala | Arg |
|---|---|---|---|---|---|---|---|---|---|---|
| P8F | 5' (C/T)TI | TTT | ACI | CA(A/G) | ACI | (C/T)TI | AAA | GAA | GCI | (A/C)G 3' |

Another set of degenerate oligonucleotides was synthesized (pool P8.108.3R), representing the complement of the coding strand for the determined amino acid sequence of the TcaB$_i$-PT108 internal peptide (disclosed herein as SEQ ID NO:20):

| Amino Acid | Met | Tyr | Tyr | Ile | Gln | Ala | Gln | Gln |
|---|---|---|---|---|---|---|---|---|

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Codons    ATG TA(T/C) TA(T/C) AT(T/C/A) CA(A/G) GC(A/C/G/T) CA(A/G  CA(A/G)
P8.108.3R 3'  AT(A/G) AT(A/G) TA(A/G/T) GT(T/C) CGI          GT(T/C) GT 5'
          TAC
```

These oligonucleotides were used as primers for PCR® using HotStart 50 Tubes™ (Molecular Bio-Products, Inc., San Diego, CA) to amplify a specific DNA fragment from genomic DNA prepared from *Photorhabdus* strain W-14 (see above). A typical reaction (50 µl) contained (bottom layer) 25 pmol of each primer pool P8F and P8.108.3R, with 2 nmol each of dATP, dCTP, dGTP, and dTTP, in 1X GeneAmp® PCR buffer, and (top layer) 230 ng of genomic template DNA, 8 nmol each of dATP, dCTP, dGTP, and dTTP, and 2.5 units of AmpliTaq® DNA polymerase, in 1X GeneAmp® PCR buffer. Amplifications were performed by 35 cycles as described for the TcaC peptide. Amplification products were analyzed by electrophoresis through 0.7% w/v SeaKem® LE agarose (FMC) in TEA buffer. A specific product of estimated size 1600 bp was observed.

Four such reactions were pooled, and the amplified DNA was extracted from a 1.0% SeaKem® LE gel by Qiaex kit as described for the TcaC peptide. The extracted DNA was used directly as the template for sequence determination (PRISM™ Sequencing Kit) using the P8F and P8.108.3R primer pools. Each reaction contained about 100 ng template DNA and 25 pmol of one primer pool, and was processed according to standard protocols as described for the TcaC peptide. An analysis of the sequence derived from extension of the P8F primers revealed the short DNA sequence (and encoded amino acid sequence):

```
GAT GCA TTG  NTT  GCT
Asp Ala Leu (Val) Ala
```

which corresponds to a portion of the N-terminal peptide sequence disclosed as SEQ ID NO:3 ($TcaB_i$).

Labeling of a $TcaB_i$-peptide gene-specific probe.

Approximately 50 ng of gel-purified $TcaB_i$ DNA fragment was labeled with $^{32}$P-dCTP as described above, and nonincorporated radioisotopes were removed by passage through a NICK Column® (Pharmacia). The specific activity of the labelled DNA was determined to be 6 x $10^9$ dpm/µg. This labeled DNA was used to

probe colony membranes prepared from members of the genomic library that had hybridized to the TcaC-peptide specific probe.

The membranes containing the 12 colonies identified in the TcaC-probe library screen (see above) were stripped of radioactive TcaC-specific label by boiling twice for approximately 30 min each time in 1 liter of 0.1X SSC plus 0.1 % SDS. Removal of radiolabel was checked with a 6 hr film exposure. The stripped membranes were then incubated with the $TcaB_i$ peptide-specific probe prepared above. The labeled DNA was denatured by boiling for 10 min, and then added to the filters that had been incubated for 1 hr in 100 ml of "minimal hyb" solution at 60°C. After overnight hybridization at this temperature, the probe solution was removed, and the filters were washed as follows (all in 0.3X SSC plus 0.1% SDS): once for 5 min at 25°C, once for 1 hr at 60°C in fresh solution, and once for 1 hr at 63°C in fresh solution. After 1.5 hr exposure to X-ray film by standard procedures, 4 strongly-hybridizing colonies were observed. These were, as with the TcaC-specific probe, isolates 22G12, 25A10, 26A5, and 26B10.

The same $TcaB_i$ probe solution was diluted with an equal volume (about 100 ml) of "minimal hyb" solution, and then used to screen the membranes containing the 800 members of the genomic library. After hybridization, washing, and exposure to X-ray film as described above, only the four cosmid clones 22G12, 25A10, 26A5, and 26B10, were found to hybridize strongly to this probe.

ISOLATION OF SUBCLONES CONTAINING GENES ENCODING TcaC AND $TcaB_i$ PEPTIDES, AND DETERMINATION OF DNA BASE SEQUENCE THEREOF:

Three hybridization-positive cosmids in strain XL1 Blue MR were grown with shaking overnight (200 rpm) at 30°C in 100 ml TB-$Amp_{100}$. After harvesting the cells by centrifugation, cosmid DNA was prepared using a commercially available kit (BIGprep™, 5 Prime 3 Prime, Inc., Boulder, CO), following the manufacturer's protocols. Only one cosmid, 26A5, was successfully isolated by this procedure. When digested with restriction enzyme EcoR 1 (NEB) and analyzed by gel electrophoresis, fragments of approximate sizes 14, 10, 8 (vector), 5, 3.3, 2.9, and 1.5 kbp were detected. A second attempt to isolate cosmid DNA from the same three strains (8 ml cultures; TB-$Amp_{100}$, 30°C) utilized a

boiling mini method (Evans G. and G. Wa 1987, "Cosmid vectors for genomic walking and rapid restriction mapping." in Guide to Molecular Cloning Techniques. Meth. Enzymology, vol. 152, S. Berger and A. Kimmel, eds., pgs. 604-610). Only one cosmid, 25A10, was successfully isolated by this method. When digested with restriction enzyme EcoR 1 (NEB) and analyzed by gel electrophoresis, this cosmid showed a fragmentation pattern identical to that previously seen with cosmid 26A5.

A 0.15 µg sample of 26A5 cosmid DNA was used to transform 50 ml of *E. coli* DH5α cells (Gibco BRL), by the supplier's protocols. A single colony isolate of that strain was inoculated into 4 ml of TB-$Amp_{100}$, and grown for 8 hr at 37°C. Chloramphenicol was added to a final concentration of 225 µg/ml, incubation was continued for another 24 hr, then cells were harvested by centrifugation and frozen at -20°C. Isolation of the 26A5 cosmid DNA was by a standard alkaline lysis miniprep (Maniatis *et al.*, *op. cit.*, p. 382), modified by increasing all volumes by 50% and with stirring or gentle mixing, rather than vortexing, at every step. After washing the DNA pellet in 70% ethanol, it was dissolved in TE containing 25 µg/ml ribonuclease A (Boehringer Mannheim).

Identification of EcoR 1 fragments hybridizing to GZ4-derived and TcaBi - probes. Approximately 0.4 µg of cosmid 25A10 (from XL1 Blue MR cells) and about 0.5 µg of cosmid 26A5 (from chloramphenicol-amplified DH5α cells) were each digested with about 15 units of EcoR 1 (NEB) for 85 min, frozen overnight, then heated at 65°C for five min, and electrophoresed in a 0.7% agarose gel (Seakem® LE, 1X TEA, 80 volts, 90 min). The DNA was stained with ethidium bromide as described above, and photographed under ultraviolet light. The EcoR 1 digest of cosmid 25A10 was a complete digestion, but the sample of cosmid 26A5 was only partially digested under these conditions. The agarose gel containing the DNA fragments was subjected to depurination, denaturation and neutralization, followed by Southern blotting onto a Magna NT nylon membrane, using a high salt (20X SSC) protocol, all as described in section 2.9 of Ausubel *et al.* (CPMB, *op. cit.*). The transferred DNA was then UV-crosslinked to the nylon membrane as before.

An TcaC-peptide specific DNA fragment corresponding to the insert of plasmid isolate GZ4 was amplified by PCR® in a 100 ml reaction volume as described previously above. The amplification products from three such reactions were pooled and were extracted from a 1% GTG® agarose gel by Qiaex kit, as described above, and quantitated by fluorometry. The gel-purified DNA (100 ng) was labeled with $^{32}$P-dCTP using the High Prime Labeling Mix (Boehringer Mannheim) as described above, to a specific activity of 6.34 x $10^8$ dpm/µg.

The $^{32}$P-labeled GZ4 probe was boiled 10 min, then added to "minimal hyb" buffer (at 1 ng/ml), and the Southern blot membrane containing the digested cosmid DNA fragments was added, and incubated for 4 hr at 60°C with gentle shaking at 50 rpm. The membrane was then washed 3 times at 25°C for about 5 min each (minimal hyb wash solution), followed by two washes for 30 min each at 60°C. The blot was exposed to film (with enhancer screens) for about 30 min at -70°C. The GZ4 probe hybridized strongly to the 5.0 kbp (apparent size) EcoR 1 fragment of both these two cosmids, 26A5 and 25A10.

The membrane was stripped of radioactivity by boiling for about 30 min in 0.1X SSC plus 0.1 % SDS, and absence of radiolabel was checked by exposure to film. It was then hybridized at 60°C for 3.5 hours with the (denatured) $TcaB_i$ probe in "minimal hyb" buffer previously used for screening the colony membranes (above), washed as described previously, and exposed to film for 40 min at -70°C with two enhancer screens. With both cosmids, the $TcaB_i$ probe hybridized lightly with the about 5.0 kbp EcoR 1 fragment, and strongly with a fragment of approximately 2.9 kbp.

The sample of cosmid 26A5 DNA previously described, (from DH5α cells) was used as the source of DNA from which to subclone the bands of interest. This DNA (2.5 µg) was digested with about 3 units of EcoR 1 (NEB) in a total volume of 30 µl for 1.5 hr, to give a partial digest, as confirmed by gel electrophoresis. Ten µg of pBC KS (+) DNA (Stratagene) were digested for 1.5 hr with 20 units of EcoR 1 in a total volume of 20 µl, leading to total digestion as confirmed by electrophoresis. Both EcoR 1-cut DNA preparations were diluted to 50 µl with water, to each an equal volume of PCI was added, the suspension was gently mixed, spun in

a microcentr e and the aqueous supernata was collected. DNA was precipitated by 150 µl ethanol, and the mixture was placed at -20°C overnight. Following centrifugation and drying, the EcoR 1-digested pBC KS (+) was dissolved in 100 µl TE; the partially digested 26A5 was dissolved in 20 µl TE. DNA recovery was checked by fluorometry.

In separate reactions, approximately 60 ng of EcoR 1-digested pBC KS(+) DNA was ligated with approximately 180 ng or 270 ng of partially digested cosmid 26A5 DNA. Ligations were carried out in a volume of 20 µl at 15°C for 5 hr, using T4 ligase and buffer from New England BioLabs. The ligation mixture, diluted to 100 µl with sterile TE, was used to transform frozen, competent DH5α cells (Gibco BRL) according to the supplier's instructions. Varying amounts (25-200 µl) of the transformed cells were plated on freshly prepared solid LB-Cam$_{35}$ medium with 1 mM IPTG and 50 mg/l X-gal. Plates were incubated at 37°C about 20 hr, then chilled in the dark for approximately 3 hr to intensify color for insert selection. White colonies were picked onto patch plates of the same composition and incubated overnight at 37°C.

Two colony lifts of each of the selected patch plates were prepared as follows. After picking white colonies to fresh plates, round Magna NT nylon membranes were pressed onto the patch plates, the membrane was lifted off, and subjected to denaturation, neutralization and UV crosslinking as described above for the library colony membranes. The crosslinked colony lifts were vigorously washed, including gently wiping off the excess cell debris with a tissue. One set was hybridized with the GZ4(TcaC) probe solution described earlier, and the other set was hybridized with the TcaB$_i$ probe solution described earlier, according to the 'minimal hyb' protocol, followed by washing and film exposure as described for the library colony membranes.

Colonies showing hybridization signals either only with the GZ4 probe, with both GZ4 and TcaB$_i$ probes, or only with the TcaB$_i$ probe, were selected for further work and cells were streaked for single colony isolation onto LB-Cam$_{35}$ media with IPTG and X-gal as before. Approximately 35 single colonies, from 16 different isolates, were picked into liquid LB-Cam$_{35}$ media and grown

overnight at 37°C; the cells were collected by centrifugation and plasmid DNA was isolated by a standard alkaline lysis miniprep according to Maniatis *et al.* (*op. cit.* p. 368). DNA pellets were dissolved in TE + 25 µg/ml ribonuclease A and DNA concentration was determined by fluorometry. The EcoR 1 digestion pattern was analyzed by gel electrophoresis. The following isolates were picked as useful. Isolate A17.2 contains religated pBC KS(+) only and was used for a (negative) control. Isolates D38.3 and C44.1 each contain only the 2.9 kbp, $TcaB_i$ -hybridizing EcoR 1 fragment inserted into pBC KS(+). These plasmids, named pDAB2000 and pDAB2001, respectively, are illustrated in Fig. 2.

Isolate A35.3 contains only the approximately 5 kbp, GZ4)-hybridizing EcoR 1 fragment, inserted into pBC KS(+). This plasmid was named pDAB2002 (also Fig. 2). These isolates provided templates for DNA sequencing.

Plasmids pDAB2000 and pDAB2001 were prepared using the BIGprep™ kit as before. Cultures (30 ml) were grown overnight in TB-$Cam_{35}$ to an $OD_{600}$ of 2, then plasmid was isolated according to the manufacturer's directions. DNA pellets were redissolved in 100 µl TE each, and sample integrity was checked by EcoR 1 digestion and gel electrophoretic analysis.

Sequencing reactions were run in duplicate, with one replicate using as template pDAB2000 DNA, and the other replicate using as template pDAB2001 DNA. The reactions were carried out using the dideoxy dye terminator cycle sequencing method, as described above for the sequencing of the GZ4/HB14 DNAs. Initial sequencing runs utilized as primers the LacZ and T7 primers described above, plus primers based on the determined sequence of the $TcaB_i$ PCR amplification product (TH1 = ATTGCAGACTGCCAATCGCTTCGG, TH12 = GAGAGTATCCAGACCGCGGATGATCTG).

After alignment and editing of each sequencing output, each was truncated to between 250 to 350 bases, depending on the integrity of the chromatographic data as interpreted by the Perkin Elmer Applied Biosystems Division SeqEd 675 software. Subsequent sequencing "steps" were made by selecting appropriate sequence for new primers. With a few exceptions, primers (synthesized as described above) were 24 bases in length with a 50% G+C composition. Sequencing by this method was carried out on both strands of the approximately 2.9 kbp EcoR 1 fragment.

To further serve as template for DNA sequencing, plasmid DNA from isolate pDAB2002 was prepared by BIGprep™ kit. Sequencing reactions were performed and analyzed as described above. Initially, a T3 primer (pBS SK (+) bases 774-796: CGCGCAATTAACCCTCACTAAAG) and a T7 primer (pBS KS (+) bases 621-643: GCGCGTAATACGACTCACTATAG) were used to prime the sequencing reactions from the flanking vector sequences, reading into the insert DNA. Another set of primers, (GZ4F: GTATCGATTACAACGCTGTCACTTCCC; TH13: GGGAAGTGACAGCGTTGTAATCGATAC; TH14: ATGTTGGGTGCGTCGGCTAATGGACATAAC; and LW1-204: GGGAAGTGACAGCGTTGTAATCGATAC) was made to prime from internal sequences, which were determined previously by degenerate oligonucleotide-mediated sequencing of subcloned TcaC-peptide PCR products. From the data generated during the initial rounds of sequencing, new sets of primers were designed and used to walk the entire length of the ~5 kbp fragment. A total of 55 oligo primers was used, enabling the identification of 4832 total bp of contiguous sequence.

When the DNA sequence of the EcoR 1 fragment insert of pDAB2002 is combined with part of the determined sequence of the pDAB2000/pDAB2001 isolates, a total contiguous sequence of 6005 bp was generated (disclosed herein as SEQ ID NO:25). When long open reading frames were translated into the corresponding amino acids, the sequence clearly shows the $TcaB_i$ N-terminal peptide (disclosed as SEQ ID NO:3), encoded by bases 19-75, immediately following a methionine residue (start of translation). Upstream lies a potential ribosome binding site (bases 1-9), and downstream, at bases 166-228 is encoded the $TcaB_i$-PT158 internal peptide (disclosed herein as SEQ ID NO:19). Further downstream, in the same reading frame, at bases 1738-1773, exists a sequence encoding the $TcaB_i$-PT108 internal peptide (disclosed herein as SEQ ID NO:20). Also in the same reading frame, at bases 1897-1923, is encoded the $TcaB_{ii}$ N-terminal peptide (disclosed herein as SEQ ID NO:5), and the reading frame continues uninterrupted to a translation termination codon at nucleotides 3586-3588.

The lack of an in-frame stop codon between the end of the sequence encoding $TcaB_i$-PT108 and the start of the $TcaB_{ii}$ encoding region, and the lack of a discernible ribosome binding site immediately upstream of the $TcaB_{ii}$ coding region, indicate that

peptides $TcaB_{ii}$ and $TcaB_i$ are encoded by a single open reading frame of 3567 bp beginning at base pair 16 in SEQ ID NO:25), and are most likely derived from a single primary gene product of 1189 amino acids (131,586 Daltons; disclosed herein as SEQ ID NO:26) by post-translational cleavage. If the amino acid immediately preceding the $TcaB_{ii}$ N-terminal peptide represents the C-terminal amino acid of peptide $TcaB_i$, then the predicted mass of $TcaB_{ii}$ (627 amino acids) is 70,814 Daltons (disclosed herein as SEQ ID NO:28), somewhat higher than the size observed by SDS-PAGE (68 kDa). This peptide would be encoded by a contiguous stretch of 1881 base pairs (disclosed herein as SEQ ID NO:27). It is thought that the native C-terminus of $TcaB_i$ lies somewhat closer to the C-terminus of $TcaB_i$-PT108. The molecular mass of PT108 [3.438 kDa; determined during N-terminal amino acid sequence analysis of this peptide] predicts a size of 30 amino acids. Using the size of this peptide to designate the C-terminus of the $TcaB_i$ coding region [Glu at position 604 of SEQ ID NO:28], the derived size of $TcaB_i$ is determined to be 604 amino acids or 68,463 Daltons, more in agreement with experimental observations.

Translation of the $TcaB_{ii}$ peptide coding region of 1686 base pairs (disclosed herein as SEQ ID NO:29) yields a protein of 562 amino acids (disclosed herein as SEQ ID NO:30) with predicted mass of 60,789 Daltons, which corresponds well with the observed 61 kDa.

A potential ribosome binding site (bases 3633-3638) is found 48 bp downstream of the stop codon for the *tcaB* open reading frame. At bases 3645-3677 is found a sequence encoding the N-terminus of peptide TcaC, (disclosed as SEQ ID NO.2). The open reading frame initiated by this N-terminal peptide continues uninterrupted to base 6005 (2361 base pairs, disclosed herein as the first 2361 base pairs of SEQ ID NO.31). A gene (*tcaC*) encoding the entire TcaC peptide, (apparent size ~165 kDa; ~1500 amino acids), would comprise about 4500 bp.

Another isolate containing cloned EcoR 1 fragments of cosmid 26A5, E20.6, was also identified by its homology to the previously mentioned GZ4 and $TcaB_i$ probes. Agarose gel analysis of EcoR 1 digests of the DNA of the plasmid harbored by this strain (pDAB2004, Fig. 2), revealed insert fragments of estimated

sizes 2.9, and 3.3 kbp. DNA sequence analysis initiated from primers designed from the sequence of plasmid pDAB2002 revealed that the 3.3 kbp EcoR I fragment of pDAB2004 lies adjacent to the 5 kbp EcoR I fragment represented in pDAB2002. The 2361 base pair open reading frame discovered in pDAB2002 continues uninterrupted for another 2094 bases in pDAB2004 [disclosed herein as base pairs 2362 to 4458 of SEQ ID NO:31]. DNA sequence analysis using the parent cosmid 26A5 DNA as template confirmed the continuity of the open reading frame. Altogether, the open reading frame (*TcaC* SEQ ID NO:31) comprises 4455 base pairs, and encodes a protein (TcaC) of 1485 amino acids [disclosed herein as SEQ ID NO:32]. The calculated molecular size of 166,214 Daltons is consistent with the estimated size of the TcaC peptide (165 kDa), and the derived amino acid sequence matches exactly that disclosed for the TcaC N-terminal sequence [SEQ ID NO:2].

The lack of an amino acid sequence corresponding to SEQ ID NO:17; used to design the degenerate oligonucleotide primer pool in the discovered sequence indicates that the generation of the PCR® products found in isolates GZ4 and HB14, which were used as probes in the initial library screen, were fortuitously generated by reverse-strand priming by one of the primers in the degenerate pool. Further, the derived protein sequence does not include the internal fragment disclosed herein as SEQ ID NO:18. These sequences reveal that plasmid pDAB2004 contains the complete coding region for the TcaC peptide.

## Example 9

## Screening of the *Photorhabdus* genomic library for genes encoding the $TcbA_{ii}$ peptide

This example describes a method used to identify DNA clones that contain the $TcbA_{ii}$ peptide-encoding genes, the isolation of the gene, and the determination of its partial DNA base sequence.

### Primers and PCR reactions

The $TcbA_{ii}$ polypeptide of the insect active preparation is ~206 kDa. The amino acid sequence of the N-terminus of this peptide is disclosed as SEQ ID NO:1. Four pools of degenerate oligonucleotide primers ("Forward primers": TH-4, TH-5, TH-6, and

TH-7) were synthesized to encode a portion of this amino acid sequence, as described in Example 8, and are shown below.

Table 11

| Amino Acid | Phe | Ile | Gln | Gly | Tyr | Ser | Asp | Leu | Phe |
|---|---|---|---|---|---|---|---|---|---|
| TH-4 | 5'-TT(T/C) | ATI | CA(A/G) | GGI | TA(T/C) | TCI | GA(T/C) | CTI | TT-3' |
| TH-5 | 5'-TT(T/C) | ATI | CA(A/G) | GGI | TA(T/C) | AG(T/C) | GA(T/C) | CTI | TT-3' |
| TH-6 | 5'-TT(T/C) | ATI | CA(A/G) | GGI | TA(T/C) | TCI | GA(T/C) | TT(A/G) | TT-3' |
| TH-7 | 5'-TT(T/C) | ATI | CA(A/G) | GGI | TA(T/C) | AG(T/C) | GA(T/C) | TT(A/G) | TT-3' |

In addition, a primary ("a") and a secondary ("b") sequence of an internal peptide preparation (TcbA$_{ii}$-PT81) have been determined and are disclosed herein as SEQ ID No:23 and SEQ ID No:24, respectively. Four pools of degenerate oligonucleotides ("Reverse Primers": TH-8, TH-9, TH-10 and TH-11) were similarly designed and synthesized to encode the reverse complement of sequences that encode a portion of the peptide of SEQ ID NO:23, as shown below.

Table 12

| Amino Acid | Thr | Tyr | Leu | Thr | Ser | Phe | Glu | Gln | Val | Ala | Asn | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TH-8 | 3'TGI | AT(A/G) | GAI | TGI | AGI | AA(A/G) | CT(T/C) | GT(T/C) | CAI | CGI | TT(G/A) | -5' |
| TH-9 | 3'TGI | AT(A/G) | TT(A/G) | TGI | AGI | AA(A/G) | CT(T/C) | GT(T/C) | CAI | CGI | TT(G/A) | -5' |
| TH-10 | 3'TGI | AT(A/G) | GAI | TGI | TC(G/A) | AA(A/G) | CT(T/C) | GT(T/C) | CAI | CGI | TT(G/A) | -5' |
| TH-11 | 3'TGI | AT(A/G) | TT(A/G) | TGI | TC(G/A) | AA(A/G) | CT(T/C) | GT(T/C) | CAI | CGI | TT(G/A) | -5' |

Sets these primers were used in PCR reactions to amplify TcbAii- encoding gene fragments from the genomic *Photorhabdus luminescens* W-14 DNA prepared in Example 6. All PCR® reactions were run with the "Hot Start" technique using AmpliWax™ gems and other Perkin Elmer reagents and protocols. Typically, a mixture (total volume 11 µl) of $MgCl_2$, dNTP's, 10X GeneAmp® PCR Buffer II, and the primers were added to tubes containing a single wax bead. [10X GeneAmp® PCR Buffer II is composed of 100 mM Tris-HCl, pH 8.3; and 500 mM KCl.] The tubes were heated to 80°C for 2 minutes and allowed to cool. To the top of the wax seals, a solution containing 10X GeneAmp® PCR Buffer II, DNA template, and AmpliTaq® DNA polymerase were added. Following melting of the wax seal and mixing of components by thermal cycling, final reaction conditions (volume of 50 µl) were: 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM $MgCl_2$; 200 µM each in dATP, dCTP, dGTP, dTTP; 1.25 mM in a single Forward primer pool; 1.25 µM in a single Reverse primer pool, 1.25 units of AmpliTaq® DNA polymerase, and 170 ng of template DNA.

The reactions were placed in a thermocycler (as in Example 8) and run with the following program:

Table 13

| Temperature | Time | Cycle Repetition |
|---|---|---|
| 94°C | 2 minutes | 1X |
| | | |
| 94°C | 15 seconds | |
| 55-65°C | 30 seconds | 30X |
| 72°C | 1 minute | |
| | | |
| 72°C | 7 minutes | 1X |
| | | |
| 15°C | Constant | |

A series of amplifications was run at three different annealing temperatures (55°, 60°, 65° C) using the degenerate primer pools. Reactions with annealing at 65°C had no amplification products visible following agarose gel electrophoresis. Reactions having a 60°C annealing regime and containing primers TH-5+TH-10 produced an amplification product that had a mobility corresponding to 2.9 kbp. A lesser amount of the 2.9 kbp product was produced under these conditions with primers TH-7+TH-10. When reactions were annealed at 55°C, these primer pairs produced more of the 2.9 kbp product, and this product was also produced by primer pairs TH-5+TH-8 and TH-5+TH-11. Additional very faint 2.9 kbp bands were seen in lanes containing amplification products from primer pairs TH-7 plus TH-8, TH-9, TH-10, or TH-11.

To obtain sufficient PCR amplification product for cloning and DNA sequence determination, 10 separate PCR reactions were set up using the primers TH-5+TH-10, and were run using the above conditions with a 55°C annealing temperature. All reactions were pooled and the 2.9 kbp product was purified by Qiaex extraction from an agarose gel as described above.

Additional sequences determined for $TcbA_{ii}$ internal peptides are disclosed herein as SEQ ID NO:21 and SEQ ID NO:22. As before, degenerate oligonucleotides (Reverse primers TH-17 and TH-18) were made corresponding to the reverse complement of sequences that encode a portion of the amino acid sequence of these peptides.

Table 14

**From SEQ ID NO:21**

| Amino Acid | Met | Glu | Thr | Gln | Asn | Ile | Gln | Glu | Pro |
|---|---|---|---|---|---|---|---|---|---|
| TH-17 | 3'-TAC | CTT/C | TGI | GTT/C | TTA/G | TAI | GTT/C | GTT/C | GG-5' |

Table 15

**From SEQ ID NO:22**

| Amino Acid | Asn | Pro | Ile | Asn | Ile | Asn | Thr | Gly | Ile | Asp |
|---|---|---|---|---|---|---|---|---|---|---|
| TH-18 | 3'-TT(A/G) | GGI | TAI | TT(A/G) | TAI | TT(A?G) | TGI | CCI | TAI | CT(A/G)-5' |

Degenerate oligonucleotides TH-18 TH-17 were used in an amplification experiment with *Photorhabdus luminescens* W-14 DNA as template and primers TH-4, TH-5, TH-6, or TH-7 as the 5'-(Forward) primers. These reactions amplified products of approximately 4 kbp and 4.5 kbp, respectively. These DNAs were transferred from agarose gels to nylon membranes and hybridized with a $^{32}$P-labeled probe (as described above) prepared from the 2.9 kbp product amplified by the TH-5+TH10 primer pair. Both the 4 kbp and the 4.5 kbp amplification products hybridized strongly to the 2.9 kbp probe. These results were used to construct a map ordering the TcbA$_{ii}$ internal peptide sequences as shown in Fig. 3. Approximate distances between the primers are shown in nucleotides in Fig. 3.

DNA Sequence of the 2.9 kbp TcbA$_{ii}$-encoding fragment

Approximately 200 ng of the purified 2.9 kbp fragment (prepared above) was precipitated with ethanol and dissolved in 17 ml of water. One-half of this was used as sequencing template with 25 pmol of the TH-5 pool as primers, the other half was used as template for TH-10 priming. Sequencing reactions were as given in Example 8. No reliable sequence was produced using the TH-10 primer pool; however, reactions with TH-5 primer pool produced the sequence disclosed below:.

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  1   AATCGTGTTG ATCCCTATGC CGNGCCGGGT TCGGTGGAAT CGATGTCCTC ACCGGGGGTT
 61   TATTNGAGGG ANTNGTCCCG TGAGGCCAAA AANTGGAATG AAAGAAGTTC AATTTNTTAC
121   CTAGATAAAC GTCGCCCGGN TTTAGAAAGN TTANTGNTCA GCCAGAAAAT TTTGGTTGAG
181   GAAATTCCAC CGNTGGTTCT CTCTATTGAT TNGGGCCTGG CCGGGTTCGA ANNAAAACNA
241   GGAAATNCAC AAGTTGAGGT GATGGNTTTG TNGCNANCTT NTCGTTTAGG TGGGGAGAAA
301   CCTTNTCANC ACGNTTNTGA AACTGTCCGG GAAATCGTCC ATGANCGTGA NCCAGGNTTN
361   CGCCATTGG
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Based on this sequence, a sequencing primer (TH-21, 5'-CCGGGCGACGTTTATCTAGG-3') was designed to reverse complement bases 120-139, and initiate polymerization towards the 5' end (i.e., TH-5 end) of the gel-purified 2.9 kbp TcbA$_{ii}$-encoding PCR fragment. The determined sequence is shown below, and is compared to the biochemically determined N-terminal peptide sequence of TcbA$_{ii}$ SEQ ID NO:1.

TcbAii 2.9 kb PCR fragment Sequence Confirmation
(Underlined amino acids = encoded by degenerate oligonucleotides)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SEQ ID NO:1 | F | I | Q | G | Y | S | D | L | F | G | - | - | A |
| 2.9 kbp seq | GC | ATG | CAG | GGG | TAT | AGT | GAC | CTG | TTT | GGT | AAT | CGT | GCT |
| | | M | Q | G | Y | S | D | L | F | G | N | R | A |

From the homology of the derived amino acid sequence to the biochemically determined one, it is clear that the 2.9 kbp PCR fragment represents the *TcbA* coding region. This 2.9 kbp fragment was then used as a hybridization probe to screen the *Photorhabdus* W-14 genomic library prepared in Example 8 for cosmids containing the $TcbA_{ii}$-encoding gene.

Screening the *Photorhabdus* cosmid library

The 2.9 kb gel-purified PCR fragment was labeled with $^{32}P$ using the Boehringer Mannheim High Prime labeling kit as described in Example 8. Filters containing remnants of approximately 800 colonies from the cosmid library were screened as described previously (Example 8), and positive clones were streaked for isolated colonies and rescreened. Three clones (8A11, 25G8, and 26D1) gave positive results through several screening and characterization steps. No hybridization of the $TcbA_{ii}$-specific probe was ever observed with any of the four cosmids identified in Example 8, and which contain the *tcaB* and *tcaC* genes. DNA from cosmids 8A11, 25G8, and 26D1 was digested with restriction enzymes Bgl 2, EcoR 1 or Hind 3 (either alone or in combination with one another), and the fragments were separated on an agarose gel and transferred to a nylon membrane as described in Example 8. The membrane was hybridized with $^{32}P$-labeled probe prepared from the 4.5 kbp fragment (generated by amplification of *Photorhabdus* genomic DNA with primers TH-5+TH-17). The patterns generated from cosmid DNAs 8A11 and 26D1 were identical to those generated with similarly-cut genomic DNA on the same membrane. It is concluded that cosmids 8A11 and 26D1 are accurate representations of the genomic $TcbA_{ii}$ encoding locus. However, cosmid 25G8 has a single Bgl 2 fragment which is slightly larger than the genomic DNA. This may result from positioning of the insert within the vector.

DNA sequence of the *tcbA*-encoding gene

The membrane hybridization analysis of cosmid 26D1 revealed that the 4.5 kbp probe hybridized to a single large EcoR 1 fragment (greater than 9 kbp). This fragment was gel purified and ligated into the EcoR 1 site of pBC KS (+) as described in Example 8, to generate plasmid pBC-S1/R1. The partial DNA sequence of the insert DNA of this plasmid was determined by "primer walking" from the flanking vector sequence, using procedures described in Example 8. Further sequence was generated by extension from new oligonucleotides designed from the previously determined sequence. When compared to the determined DNA sequence for the *tcbA* gene identified by other methods (disclosed herein as SEQ ID NO:11 as described in Example 12 below), complete homology was found to nucleotides 1-272, 319-826, 2578-3036, and 3068-3540 (total bases = 1712). It was concluded that both approaches can be used to identify DNA fragments encoding the $TcbA_{ii}$ peptide.

Analysis of the derived amino acid sequence of the *tcbA* gene.

The sequence of the DNA fragment identified as SEQ ID NO:11 encodes a protein whose derived amino acid sequence is disclosed herein as SEQ ID NO:12. Several features verify the identity of the gene as that encoding the $TcbA_{ii}$ protein. The $TcbA_{ii}$ N-terminal peptide (SEQ ID NO:1; Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala) is encoded as amino acids 88-100. The $TcbA_{ii}$ internal peptide $TcbA_{ii}$-PT81(a) (SEQ ID NO:23) is encoded as amino acids 1065-1077, and $TcbA_{ii}$-PT81(b) (SEQ ID NO:24) is encoded as amino acids 1571-1592. Further, the internal peptide $TcbA_{ii}$-PT56 (SEQ ID NO:22) is encoded as amino acids 1474-1488, and the internal peptide $TcbA_{ii}$-PT103 (SEQ ID NO:24) is encoded as amino acids 1614-1639. It is obvious that this gene is an authentic clone encoding the $TcbA_{ii}$ peptide as isolated from insecticidal protein preparations of *Photorhabdus luminescens* strain W-14.

The protein isolated as peptide $TcbA_{ii}$ is derived from cleavage of a longer peptide. Evidence for this is provided by the fact that the nucleotides encoding the $TcbA_{ii}$ N-terminal peptide SEQ ID NO:1 are preceded by 261 bases (encoding 87 N-terminal-proximal amino acids) of a longer open reading frame (SEQ ID NO:11). This reading frame begins with nucleotides that encode the amino acid sequence Met Gln Asn Ser

Leu, which corresponds to the N-terminal sequence of the large peptide TcbA, and is disclosed herein as SEQ ID NO:16. It is thought that TcbA is the precursor protein for TcbAii.

Relationship of *tcbA*, *tcaB* and *tcaC* genes.

The *tcaB* and *tcaC* genes are closely linked and may be transcribed as a single mRNA (Example 8). The *tcbA* gene is borne on cosmids that apparently do not overlap the ones harboring the *tcaB* and *tcaC* cluster, since the respective genomic library screens identified different cosmids. However, comparison of the amino sequences encoded by the *tcaB* and *tcaC* genes with the *tcbA* gene reveals a substantial degree of homology. The amino acid conservation (Protein Alignment Mode of MacVector™ Sequence Analysis Software, scoring matrix pam250, hash value = 2; Kodak Scientific Imaging Systems, Rochester, NY) is shown in Fig. 4. On the score line of each panel in Fig. 4, up carats (^) indicate homology or conservative amino acid changes,and down carats (v) indicate nonhomology.

This analysis shows that the amino acid sequence of the TcbA peptide from residues 1739 to 1894 is highly homologous to amino acids 441 to 603 of the $TcaB_i$ peptide (162 of the total 627 amino acids of P8; SEQ ID NO:28). In addition, the sequence of TcbA amino acids 1932 to 2459 is highly homologous to amino acids 12 to 531 of peptide $TcaB_{ii}$ (520 of the total 562 amino acids; SEQ ID NO:30). Considering that the TcbA peptide (SEQ ID NO:12) comprises 2505 amino acids, a total of 684 amino acids (27%) at the C-proximal end of it is homologous to the $TcaB_i$ or $TcaB_{ii}$ peptides, and the homologies are arranged colinear to the arrangement of the putative TcaB preprotein (SEQ ID NO:26). A sizeable gap in the TcbA homology coincides with the junction between the $TcaB_i$ and $TcaB_{ii}$ portions of the TcaB preprotein. Clearly the TcbA and TcaB gene products are evolutionarily related, and it is proposed that they share some common function(s) in *Photorhabdus*.

## Example 10

## Characterization of zinc-metalloproteases in *Photorhabdus* Broth: Protease Inhibition, Classification, and Purification

Protease Inhibition and Classification Assays: Protease assays were performed using FITC-casein dissolved in water as substrate (0.08% final assay concentration). Proteolysis reactions were performed at 25°C for 1 h in the appropriate buffer with 25 µl of *Photorhabdus* broth (150 µl total reaction volume). Samples were also assayed in the presence and absence of dithiothreitol. After incubation, an equal volume of 12% trichloroacetic acid was added to precipitate undigested protein. Following precipitation for 0.5 h and subsequent centrifugation, 100 µl of the supernatant was placed into a 96-well microtiter plate and the pH of the solution was adjusted by addition of an equal volume of 4N NaOH. Proteolysis was then quantitated using a Fluoroskan II fluorometric plate reader at excitation and emission wavelengths of 485 and 538 nm, respectively. Protease activity was tested over a range from pH 5.0-10.0 in 0.5 units increments. The following buffers were used at 50 mM final concentration: sodium acetate (pH 5.0 - 6.5); Tris-HCL (pH 7.0 - 8.0); and bis-Tris propane (pH 8.5-10.0). To identify the class of protease(s) observed, crude broth was treated with a variety of protease inhibitors (0.5 µg/µl final concentration) and then examined for protease activity at pH 8.0 using the substrate described above. The protease inhibitors used included E-64 (L-trans-expoxysaccinylleucylamido[4-,-guanidino]-butane), 3,4 dichloroisocoumarin, Leupeptin, pepstatin, amastatin, ethylenediaminetetraacetic acid (EDTA) and 1,10 phenanthroline.

Protease assays performed over a pH range revealed that indeed protease(s) were present which exhibited maximal activity at ~ pH 8.0 (Table 16). Addition of DTT did not have any effect on protease activity. Crude broth was then treated with a variety of protease inhibitors (Table 17). Treatment of crude broth with the inhibitors described above revealed that 1,10 phenanthroline caused complete inhibition of all protease activity when added at a final concentration of 50 µg, with the $IC_{50}$ = 5 µg in 100 µl of a 2 mg/ml crude broth solution. These data indicate that the most abundant protease(s) found in the

*Photorhabdus* b[redacted]n are from the zinc-metallo ease class of enzymes.

Table 16

Effect of pH on the protease activity found in a Day 1 production of *Photorhabdus luminescens* (strain W-14).

| pH | Flu. Units[a] Activity[b] | Percent |
|---|---|---|
| 5.0 | 3013 ± 78 | 17 |
| 5.5 | 7994 ± 448 | 45 |
| 6.0 | 12965 ± 483 | 74 |
| 6.5 | 14390 ± 1291 | 82 |
| 7.0 | 14386 ± 1287 | 82 |
| 7.5 | 14135 ± 198 | 80 |
| 8.0 | 17582 ± 831 | 100 |
| 8.5 | 16183 ± 953 | 92 |
| 9.0 | 16795 ± 760 | 96 |
| 9.5 | 16279 ± 1022 | 93 |
| 10.0 | 15225 ± 210 | 87 |

a Flu. Units = Fluorescence Units (Maximum = ~28,000; background = ~ 2200).

b. Percent activity relative to the maximum at pH 8.0

Table 17
Effect of different protease inhibitors on the protease activity at pH 8 found in a Day 1 production of *Photorhabdus luminescens* (strain W-14).

| Inhibitor | Corrected Flu. Units[a] | Percent Inhibition[b] |
|---|---|---|
| Control | 13053 | 0 |
| E-64 | 14259 | 0 |
| 1,10 Phenanthroline[c] | 15 | 99 |
| 3,4 Dichloroisocoumarin[d] | 7956 | 39 |
| Leupeptin | 13074 | 0 |
| Pepstatin[c] | 13441 | 0 |
| Amastatin | 12474 | 4 |
| DMSO Control | 12005 | 8 |
| Methanol Control | 12125 | 7 |

a. Corrected Flu. Units = Fluorescence Units - background(2200 flu. units).
b. Percent Inhibition relative to protease activity at pH 8.0.
c. Inhibitors were dissolved in methanol.
d. Inhibitors were dissolved in DMSO.

The isolation of a zinc-metalloprotease was performed by applying dialyzed 10-80% ammonium sulfate pellet to a Q Sepharose column equilibrated at 50 mM $Na_2PO_4$, pH 7.0 as described in Example 5 for *Photorhabdus* toxin. After extensive washing, a 0 to 0.5 M NaCl gradient was used to elute toxin protein. The majority of biological activity and protein was eluted from 0.15 - 0.45 M NaCl. However, it was observed that the majority of proteolytic activity was present in the 0.25-0.35 M NaCl fraction with some activity in the 0.15-0.25 M NaCl fraction. SDS PAGE analysis of the 0.25-0.35 M NaCl fraction showed a major peptide band of approximately 60 kDa. The 0.15-0.25 M NaCl fraction contained a similar 60 kDa band but at lower relative protein concentration. Subsequent gel filtration of this fraction using a Superose 12 HR 16/50 column resulted in a major peak migrating at 57.5 kDa that contained a predominant (> 90% of total stained protein) 58.5 kDa band by SDS PAGE analysis. Additional analysis of this fraction using various protease inhibitors as described above determined that the protease was a zinc-metalloprotease. Nearly all of the protease activity present in *Photorhabdus* broth at day 1 of fermentation corresponded to the ~58 kDa zinc-metalloprotease.

In yet a second isolation of zinc-metalloprotease(s), W-14 *Photorhabdus* broth grown for three days was taken and protease

activity was ialized using sodium dodecy lfate-polyacrylamide gel electrophoresis (SDS-PAGE) laced with gelatin as described in Schmidt, T.M., Bleakley, B. and Nealson, K.M. 1988. SDS running gels (5.5 x 8 cm) were made with 12.5 % polyacrylamide (40% stock solution of acrylamide/bis-acrylamide; Sigma Chemical Co., St. Louis, MO) into which 0.1% gelatin final concentration (Biorad EIA grade reagent; Richmond CA) was incorporated upon dissolving in water. SDS-stacking gels (1.0 x 8 cm) were made with 5% polyacrylamide, also laced with 0.1% gelatin. Typically, 2.5 µg of protein to be tested was diluted in 0.03 ml of SDS-PAGE loading buffer without dithiothreitol (DTT) and loaded onto the gel. Proteins were electrophoresed in SDS running buffer (Laemmli, U.K. 1970. Nature 227, 680) at 0° C and at 8 mA. After electrophoresis was complete, the gel was washed for 2 h in 2.5% (v/v) Triton X-100. Gels were then incubated for 1 h at 37 °C in 0.1 M glycine (pH 8.0). After incubation, gels were fixed and stained overnight with 0.1% amido black in methanol-acetic acid- water (30:10:60, vol./vol./vol.; Sigma Chemical Co.). Protease activity was visualized as light areas against a dark, amido black stained background due to proteolysis and subsequent diffusion of incorporated gelatin. At least three distinct bands produced by proteolytic activity at 58-, 41-, and 38 kDa were observed.

Activity assays of the different proteases in W-14 day three culture broth were performed using FITC-casein dissolved in water as substrate (0.02% final assay concentration). Proteolysis experiments were performed at 37 °C for 0-0.5 h in 0.1M Tris-HCl (pH 8.0) with different protein fractions in a total volume of 0.15 ml. Reactions were terminated by addition of an equal volume of 12% trichloroacetic acid (TCA) dissolved in water. After incubation at room temperature for 0.25 h, samples were centrifuged at 10,000 x g for 0.25 h and 0.10 ml aliquots were removed and placed into 96-well microtiter plates. The solution was then neutralized by the addition of an equal volume of 2 N sodium hydroxide, followed by quantitation using a Fluoroskan II fluorometric plate reader with excitation and emission wavelengths of 485 and 538 nm, respectively. Activity measurements were performed using FITC-Casein with different protease concentrations at 37° C for 0-10 min. A unit of

activity was arbitrarily defined as the amount of enzyme needed to produce 1000 fluorescent units/min and specific activity was defined as units/mg of protease.

Inhibition studies were performed using two zinc-metalloprotease inhibitors; 1,10 phenanthroline and N-(a-rhamnopyranosyloxyhydroxyphosphinyl)-Leu-Trp(phosphoramidon) with stock solutions of the inhibitors dissolved in 100% ethanol and water, respectively. Stock concentrations were typically 10 mg/ml and 5 mg/ml for 1,10 phenanthroline and phosphoramidon, respectively, with final concentrations of inhibitor at 0.5-1.0 mg/ml per reaction. Treatment of three day W-14 crude broth with 1,10 phenanthroline, an inhibitor of all zinc metalloproteases, resulted in complete elimination of all protease activity while treatment with phosphoramidon, an inhibitor of thermolysin-like proteases (Weaver, L.H., Kester, W.R., and Matthews, B.W. 1977. J. Mol. Biol. 114, 119-132), resulted in ~56% reduction of protease activity. The residual proteolytic activity could not be further reduced with additional phosphoramidon.

The proteases of three day W-14 *Photorhabdus* broth were purified as follows: 4.0 liters of broth were concentrated using an Amicon spiral ultra filtration cartridge Type S1Y100 attached to an Amicon M-12 filtration device. The flow-through material having native proteins less than 100 kDa in size (3.8 L) was concentrated to 0.375 L using an Amicon spiral ultra filtration cartridge Type S1Y10 attached to an Amicon M-12 filtration device. The retentate material contained proteins ranging in size from 10-100 kDa. This material was loaded onto a Pharmacia HR16/10 column which had been packed with PerSeptive Biosystem (Framington, MA) Poros® 50 HQ strong anion exchange packing that had been equilibrated in 10 mM sodium phosphate buffer (pH 7.0). Proteins were loaded on the column at a flow rate of 5 ml/min, followed by washing unbound protein with buffer until $A_{280}$ = 0.00. Afterwards, proteins were eluted using a NaCl gradient of 0-1.0 M NaCl in 40 min at a flow rate of 7.5 ml/min. Fractions were assayed for protease activity, *supra.*, and active fractions were pooled. Proteolytically active fractions were diluted with 50% (v/v) 10 mM sodium phosphate buffer (pH 7.0) and loaded onto a Pharmacia HR 10/10 Mono Q column equilibrated in 10 mM sodium phosphate. After washing the column with buffer until $A_{280}$ =

0.00, protein were eluted using a NaCl gradient of 0-0.5 M NaCl for 1 h at a flow rate of 2.0 ml/min. Fractions were assayed for protease activity. Those fractions having the greatest amount of phosphoramidon-sensitive protease activity, the phosphoramidon sensitive activity being due to the 41/38 kDa protease, *infra.*, were pooled. These fractions were found to elute at a range of 0.15-0.25 M NaCl. Fractions containing a predominance of phosphoramidon-insensitive protease activity, the 58 kDa protease, were also pooled. These fractions were found to elute at a range of 0.25-0.35 M NaCl. The phosphoramidon-sensitive protease fractions were then concentrated to a final volume of 0.75 ml using a Millipore Ultrafree®-15 centrifugal filter device Biomax-5K NMWL membrane. This material was applied at a flow rate of 0.5 ml/min to a Pharmacia HR 10/30 column that had been packed with Pharmacia Sephadex G-50 equilibrated in 10 mM sodium phosphate buffer (pH 7.0)/ 0.1 M NaCl. Fractions having the maximal phosphoramidon-sensitive protease activity were then pooled and centrifuged over a Millipore Ultrafree®-15 centrifugal filter device Biomax-50K NMWL membrane. Proteolytic activity analysis, *supra.*, indicated this material to have only phosphoramidon-sensitive protease activity. Pooling of the phosphoramidon-insensitive protease, the 58 kDa protein, was followed by concentrating in a Millipore Ultrafree®-15 centrifugal filter device Biomax-50K NMWL membrane and further separation on a Pharmacia Superdex-75 column. Fractions containing the protease were pooled.

Analysis of purified 58- and 41/38 kDa purified proteases revealed that, while both types of protease were completely inhibited with 1,10 phenanthroline, only the 41/38 kDa protease was inhibited with phosphoramidon. Further analysis of crude broth indicated that protease activity of day 1 W-14 broth has 23% of the total protease activity due to the 41/38 kDa protease, increasing to 44% in day three W-14 broth.

Standard SDS-PAGE analysis for examining protein purity and obtaining amino terminal sequence was performed using 4-20% gradient MiniPlus SepraGels purchased from Integrated Separation Systems (Natick, MA). Proteins to be amino-terminal sequenced were blotted onto PVDF membrane following purification, *infra.*, (ProBlott™ Membranes; Applied Biosystems, Foster City, CA),

visualized with 0.1% amido black, excised, and sent to Cambridge Prochem; Cambridge, MA, for sequencing.

Deduced amino terminal sequence of the 58- (SEQ ID NO:45) and 41/38 kDa (SEQ ID NO:44) proteases from three day old W-14 broth were DV-GSEKANEKLK (SEQ ID NO: 45) and DSGDDDKVTNTDIHR (SEQ ID NO:44), respectively.

Sequencing of the 41/38 kDa protease revealed several amino termini, each one having an additional amino acid removed by proteolysis. Examination of the primary, secondary, tertiary and quartenary sequences for the 38 and 41 kDa polypeptides allowed for deduction of the sequence shown above and revealed that these two proteases are homologous.

## Example 11, Part A

## Screening of *Photorhabdus* Genomic Library via use of Antibodies for Genes encoding TcbA Peptide

In parallel to the sequencing described above, suitable probing and sequencing was done based on the TcbA$_{ii}$ peptide (SEQ ID NO:1). This sequencing was performed by preparing bacterial culture broths and purifying the toxin as described in Examples 1 and 2 above.

Genomic DNA was isolated from the *Photorhabdus luminescens* strain W-14 grown in Grace's insect tissue culture medium. The bacteria were grown in 5 ml of culture medium in a 250 ml Erlenmeyer flask at 28°C and 250 rpm for approximately 24 hours. Bacterial cells from 100 ml of culture medium were pelleted at 5000 x g for 10 minutes. The supernatant was discarded, and the cell pellets then were used for the genomic DNA isolation.

The genomic DNA was isolated using a modification of the CTAB method described in Section 2.4.3 of Ausubel (*supra.*). The section entitled "Large Scale CsCl prep of bacterial genomic DNA" was followed through step 6. At this point, an additional chloroform/isoamyl alcohol (24:1) extraction was performed followed by a phenol/chloroform/isoamyl (25:24:1) extraction step and a final chloroform/isoamyl/alcohol (24:1) extraction. The DNA was precipitated by the addition of a 0.6 volume of isopropanol. The precipitated DNA was hooked and wound around the end of a bent glass rod, dipped briefly into 70% ethanol as a final wash, and dissolved in 3 ml of TE buffer.

The DNA concentration, estimated by optical density at 280/260 nm, was approximately 2 mg/ml.

Using this genomic DNA, a library was prepared. Approximately 50 µg of genomic DNA was partly digested with Sau3 A1. Then NaCl density gradient centrifugation was used to size fractionate the partially digested DNA fragments. Fractions containing DNA fragments with an average size of 12 kb, or larger, as determined by agarose gel electrophoresis, were ligated into the plasmid BluScript, Stratagene, La Jolla, California, and transformed into an *E. coli* DH5α or DHB10 strain.

Separately, purified aliquots of the protein were sent to the biotechnology hybridoma center at the University of Wisconsin, Madison for production of monoclonal antibodies to the proteins. The material that was sent was the HPLC purified fraction containing native bands 1 and 2 which had been denatured at 65°C, and 20 µg of which was injected into each of four mice. Stable monoclonal antibody-producing hybridoma cell lines were recovered after spleen cells from unimmunized mouse were fused with a stable myeloma cell line. Monoclonal antibodies were recovered from the hybridomas.

Separately, polyclonal antibodies were created by taking native agarose gel purified band 1 (see Example 1) protein which was then used to immunize a New Zealand white rabbit. The protein was prepared by excising the band from the native agarose gels, briefly heating the gel pieces to 65°C to melt the agarose, and immediately emulsifying with adjuvant. Freund's complete adjuvant was used for the primary immunizations and Freund's incomplete was used for 3 additional injections at monthly intervals. For each injection, approximately 0.2 ml of emulsified band 1, containing 50 to 100 micrograms of protein, was delivered by multiple subcontaneous injections into the back of the rabbit. Serum was obtained 10 days after the final injection and additional bleeds were performed at weekly intervals for 3 weeks. The serum complement was inactivated by heating to 56°C for 15 minutes and then stored at -20°C.

The monoclonal and polyclonal antibodies were then used to screen the genomic library for the expression of antigens which could be detected by the epitope. Positive clones were detected on nitrocellulose filter colony lifts. An immunoblot analysis of the positive clones was undertaken.

An ysis of the clones as defin[redacted]by both immunoblot and Southern analysis resulted in the tentative identification of five classes of clones.

In the first class of clone was a gene encoding the peptide designated here as $TcbA_{ii}$. Full DNA sequence of this gene (*TcbA*) was obtained. It is set forth as SEQ ID NO:11. Confirmation that the sequence encodes the internal sequence of SEQ ID NO:1 is demonstrated by the presence of SEQ ID NO:1 at amino acid number 88 from the deduced amino acid sequence created by the open reading frame of SEQ ID NO:11. This can be confirmed by referring to SEQ ID NO:12, which is the deduced amino acid sequence created by SEQ ID NO:11.

The second class of toxin peptides contains the segments referred to above as $TcaB_i$, $TcaB_{ii}$ and TcaC. Following the screening of the library with the polyclonal antisera, this second class of toxin genes was identified by several clones which produced different size proteins, all of which cross-reacted with the polyclonal antibody on an immunoblot and were also found to share DNA homology on a Southern Blot. Sequence comparison revealed that they belonged to the gene complex designated *TcaB* and *TcaC* above.

Three other classes of antibody toxin clones were also isolated in the polyclonal screen. These classes produced proteins that cross-react with a polyclonal antibody and also shared DNA homology with the classes as determined by Southern blotting. The classes have been designated Class III, Class IV and Class V. It was also possible to identify monoclonals that cross-reacted with Class I, II, III, and IV. This suggests that all have regions of high protein homology. Thus, it appears that the *P. luminescens* extracellular protein genes represent a family of genes which are evolutionarily related.

To further pursue the concept that there might be evolutionarily related variations in the toxin peptides contained within this organism, two approaches have been undertaken to examine other strains of *P. luminescens* for the presence of related proteins. This was done both by PCR amplification of genomic DNA and by immunoblot analysis using the polyclonal and monoclonal antibodies.

The resu indicate that related prote are produced by *P. luminescens* strains WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-11, WX-12, WX-15 and W-14.

## Example 11, Part B

## Sequence and anaylsis of Class III toxin clones - *tcc*

Further DNA sequencing was performed on plasmids isolated from Class III *E. coli* clones described in Example 11, Part A. The nucleotide sequence was shown to be three closely linked open reading frames at this genomic locus. This locus was designated *tcc* with the three open reading frames designated *tccA* SEQ ID NO:56, *tccB* SEQ ID NO:58 and *tccC* SEQ ID NO:60 (Fig. 6B).

The deduced amino acid from the *tccA* open reading frame indicates the gene encodes a protein of 105,459 Da. This protein was designated TccA. The first 12 amino acids of this protein match the N-terminal sequence obtained from a 108 kDa protein, SEQ ID NO:7, previously identified as part of the toxin complex.

The deduced amino acid from the *tccB* open reading frame indicates this gene encodes a protein of 175,716 Da. This protein was designated TccB. The first 11 amino acids of this protein match the N-terminal sequence obtained from a protein with estimated molecular weight of 185 kDa, SEQ ID NO:8.

The deduced amino acid sequence of *tccC* indicated that this open reading frame encodes a protein of 111,694 Da and the protein product was designated TccC.

## Example 12

## Characterization of *Photorhabdus* Strains

In order to establish that the collection described herein was comprised of *Photorhabdus* strains, the strains herein were assessed in terms of recognized microbiological traits that are characteristic of *Photorhabdus* and which differentiate it from other *Enterobacteriaceae* and *Xenorhabdus* spp. (Farmer, J.J. 1984. Bergey's Manual of Systemic Bacteriology, vol 1. pp. 510-511. (ed. Kreig N.R. and Holt, J.G.). Williams & Wilkins, Baltimore.; Akhurst and Boemare, 1988, Boemare et al., 1993). These characteristic traits are as follows: Gram's stain negative

rods, organism size of 0.5-2 μm in width and 2-10 um in length, red/yellow colony pigmentation, presence of crystalline inclusion bodies, presence of catalase, inability to reduce nitrate, presence of bioluminescence, ability to take up dye from growth media, positive for protease production, growth-temperature range below 37°C, survival under anaerobic conditions and positively motile. (Table 18). Reference *Escherichia coli*, *Xenorhabdus* and *Photorhabdus* strains were included in all tests for comparison. The overall results are consistent with all strains being part of the family *Enterobacteriaceae* and the genus *Photorhabdus*.

A luminometer was used to establish the bioluminescence of each strain and provide a quantitative and relative measurement of light production. For measurement of relative light emitting units, the broths from each strain (cells and media) were measured at three time intervals after inoculation in liquid culture (6, 12, and 24 hr) and compared to background luminosity (uninoculated media and water). Prior to measuring light emission from the various broths, cell density was established by measuring light absorbance (560 nM) in a Gilford Systems (Oberlin, OH) spectrophotometer using a sipper cell. Appropriate dilutions were then made (to normalize optical density to 1.0 unit) before measuring luminosity. Aliquots of the diluted broths were then placed into cuvettes (300 μl each) and read in a Bio-Orbit 1251 Luminometer (Bio-Orbit Oy, Twiku, Finland). The integration period for each sample was 45 seconds. The samples were continuously mixed (spun in baffled cuvettes) while being read to provide oxygen availability. A positive test was determined as being ≥ 5-fold background luminescence (~5-10 units). In addition, colony luminosity was detected with photographic film overlays and visually, after adaptation in a darkroom. The Gram's staining characteristics of each strain were established with a commercial Gram's stain kit (BBL, Cockeysville, MD) used in conjunction with Gram's stain control slides (Fisher Scientific, Pittsburgh, PA). Microscopic evaluation was then performed using a Zeiss microscope (Carl Zeiss, Germany) 100X oil immersion objective lens (with 10X ocular and 2X body magnification). Microscopic examination of individual strains for organism size, cellular description and inclusion bodies (the latter after logarithmic growth) was

performed using wet mount slides (10X ocular, 4X body and 40X objective magnification) with oil immersion and phase contrast microscopy with a micrometer (Akhurst, R.J. and Boemare, N.E. 1990. Entomopathogenic Nematodes in Biological Control (ed. Gaugler, R. and Kaya, H.). pp. 75-90. CRC Press, Boca Raton, USA.; Baghdiguian S., Boyer-Giglio M.H., Thaler, J.O., Bonnot G., Boemare N. 1993. Biol. Cell 79, 177-185.). Colony pigmentation was observed after inoculation on Bacto nutrient agar, (Difco Laboratories, Detroit, MI) prepared as per label instructions. Incubation occurred at 28°C and descriptions were produced after 5-7 days. To test for the presence of the enzyme catalase, a colony of the test organism was removed on a small plug from a nutrient agar plate and placed into the bottom of a glass test tube. One ml of a household hydrogen peroxide solution was gently added down the side of the tube. A positive reaction was recorded when bubbles of gas (presumptive oxygen) appeared immediately or within 5 seconds. Controls of uninoculated nutrient agar and hydrogen peroxide solution were also examined. To test for nitrate reduction, each culture was inoculated into 10 ml of Bacto Nitrate Broth (Difco Laboratories, Detroit, MI). After 24 hours incubation at 28°C, nitrite production was tested by the addition of two drops of sulfanilic acid reagent and two drops of alpha-naphthylamine reagent (see Difco Manual, 10th edition, Difco Laboratories, Detroit, MI, 1984). The generation of a distinct pink or red color indicates the formation of nitrite from nitrate. The ability of each strain to uptake dye from growth media was tested with Bacto MacConkey agar containing the dye neutral red; Bacto Tergitol-7 agar containing the dye bromothymol blue and Bacto EMB Agar containing the dye eosin-Y (agars from Difco Laboratories, Detroit, MI, all prepared according to label instructions). After inoculation on these media, dye uptake was recorded after incubation at 28°C for 5 days. Growth on these latter media is characteristic for members of the family *Enterobacteriaceae*. Motility of each strain was tested using a solution of Bacto Motility Test Medium (Difco Laboratories, Detroit, MI) prepared as per label instructions. A butt-stab inoculation was performed with each strain and motility was judged macroscopically by a diffuse zone of growth spreading from the line of inoculum. In many cases, motility was also

observed microscopically from liquid culture under wet mount slides. Biochemical nutrient evaluation for each strain was performed using BBL Enterotube II (Benton, Dickinson, Germany). Product instructions were followed with the exception that incubation was carried out at 28°C for 5 days. Results were consistent with previously cited reports for *Photorhabdus*. The production of protease was tested by observing hydrolysis of gelatin using Bacto gelatin (Difco Laboratories, Detroit, MI) plates made as per label instructions. Cultures were inoculated and the plates were incubated at 28°C for 5 days. To assess growth at different temperatures, agar plates [2% proteose peptone #3 with two percent Bacto-Agar (Difco, Detroit, MI) in deionized water] were streaked from a common source of inoculum. Plates were sealed with Nesco® film and incubated at 20, 28 and 37°C for up to three weeks. Plates showing no growth at 37°C showed no cell viability after transfer to a 28°C incubator for one week. Oxygen requirements for *Photorhabdus* strains were tested in the following manner. A butt-stab inoculation into fluid thioglycolate broth medium (Difco, Detroit, MI) was made. The tubes were incubated at room temperature for one week and cultures were then examined for type and extent of growth. The indicator resazurin demonstrates the level of medium oxidation or the aerobiosis zone (Difco Manual, 10th edition, Difco Laboratories, Detroit, MI). Growth zone results obtained for the *Photorhabdus* strains tested were consistent with those of a facultative anaerobic microorganism.

Table 18

Taxonomic Traits of *Photorhabdus* Strains

| | Traits Assessed* | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q |
| W-14 | -† | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| WX-1 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| WX-2 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| WX-3 | - | + | + | rd S | + | - | + | + | + | YT | + | + | + | + | + | + | - |
| WX-4 | - | + | + | rd S | + | - | + | + | + | YT | + | + | + | + | + | + | - |
| WX-5 | - | + | + | rd S | + | - | + | + | + | LO | + | + | + | + | + | + | - |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WX-6 | - | [redacted] | + | rd S | + | - | + | + | + | LY | [redacted] | + | + | + | + | - | - |
| WX-7 | - | + | + | rd S | + | - | + | + | + | R | + | + | + | + | + | + | - |
| WX-8 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| WX-9 | - | + | + | rd S | + | - | + | + | + | YT | + | + | + | + | + | + | - |
| WX-10 | - | + | + | rd S | + | - | + | + | + | Ro | + | + | + | + | + | + | - |
| WX-11 | - | + | + | rd S | + | - | + | + | + | Ro | + | + | + | + | + | + | - |
| WX-12 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| WX-14 | - | + | + | rd S | + | - | + | + | + | LR | + | + | + | + | + | + | - |
| WX-15 | - | + | + | rd S | + | - | + | + | + | LR | + | + | + | + | + | + | - |
| H9 | - | + | + | rd S | + | - | + | + | + | LY | + | + | + | + | + | + | - |
| Hb | - | + | + | rd S | + | - | + | + | + | YT | + | + | + | + | + | + | - |
| Hm | - | + | + | rd S | + | - | + | + | + | TY | + | + | + | + | + | + | - |
| HP88 | - | + | + | rd S | + | - | + | + | + | LY | + | + | + | + | + | + | - |
| NC-1 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| W30 | - | + | + | rd S | + | - | + | + | + | YT | + | + | + | + | + | + | - |
| WIR | - | + | + | rd S | + | - | + | + | + | RO | + | + | + | + | + | + | - |
| B2 | - | + | + | rd S | + | - | + | + | + | R | + | + | + | + | + | + | - |
| 43948 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| 43949 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| 43950 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| 43951 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |
| 43952 | - | + | + | rd S | + | - | + | + | + | O | + | + | + | + | + | + | - |

* - A = Gram's stain, B=Crystaline inclusion bodies, C=Bioluminescence, D=Cell form, E=Motility, F=Nitrate reduction, G=Presence of catalase, H=Gelatin hydrolysis, I=Dye uptake, J=Pigmentation, K=Growth on EMB agar, L=Growth on MacConkey agar, M=Growth on Tergitol-7 agar, N=Facultative anaerobe, O=Growth at 20°C, P=Growth at 28°C, Q=Growth at 37°C, + - +/- = positive or negative for trait, rd=rod, S=sized within Genus descriptors, RO=red-orange, LR = light red, R= red, O= organge, Y= yellow, T= tan, LY= light yellow, YT= yellow tan, and LO= light orange.

Cellular fatty acid analysis is a recognized tool for bacterial characterization at the genus and species level (Tornabene, T.G. 1985. Lipid Analysis and the Relationship to

Chemotaxonomy in Methods in Microbiology, Vol 18, 209-234.; Goodfellow, M. and O'Donnell, A.G. 1993. Roots of Bacterial Systematics in Handbook of New Bacterial Systematics (ed. Goodfellow, M. & O'Donnell, A.G.) pp. 3-54. London: Academic Press Ltd.), these references are incorporated herein by reference, and were used to confirm that our collection was related at the genus level. Cultures were shipped to an external, contract laboratory for fatty acid methyl ester analysis (FAME) using a Microbial ID (MIDI, Newark, DE, USA) Microbial Identification System (MIS). The MIS system consists of a Hewlett Packard HP5890A gas chromatograph with a 25mm x 0.2mm 5% methylphenyl silicone fused silica capillary column. Hydrogen is used as the carrier gas and a flame-ionization detector functions in conjunction with an automatic sampler, integrator and computer. The computer compares the sample fatty acid methyl esters to a microbial fatty acid library and against a calibration mix of known fatty acids. As selected by the contract laboratory, strains were grown for 24 hours at 28°C on trypticase soy agar prior to analysis. Extraction of samples was performed by the contract lab as per standard FAME methodology. There was no direct identification of the strains to any luminescent bacterial group other than *Photorhabdus*. When the cluster analysis was performed, which compares the fatty acid profiles of a group of isolates, the strain fatty acid profiles were related at the genus level.

The evolutionary diversity of the *Photorhabdus* strains in our collection was measured by analysis of PCR (Polymerase Chain Reaction) mediated genomic fingerprinting using genomic DNA from each strain. This technique is based on families of repetitive DNA sequences present throughout the genome of diverse bacterial species (reviewed by Versalovic, J., Schneider, M., DE Bruijn, F.J. and Lupski, J.R. 1994. Methods Mol. Cell. Biol., 5, 25-40.). Three of these, repetitive extragenic palindromic sequence (REP), enterobacterial repetitive intergenic consensus (ERIC) and the BOX element are thought to play an important role in the organization of the bacterial genome. Genomic organization is believed to be shaped by selection and the differential dispersion of these elements within the genome of closely related bacterial strains can be used to discriminate these strains (e.g.

Louws, F.J., Fulbright, D.W., Stephens, C.T. and DE Bruijn, F.J. 1994. Appl. Environ. Micro. 60, 2286-2295.). Rep-PCR utilizes oligonucleotide primers complementary to these repetitive sequences to amplify the variably sized DNA fragments lying between them. The resulting products are separated by electrophoresis to establish the DNA "fingerprint" for each strain.

To isolate genomic DNA from our strains, cell pellets were resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a final volume of 10 ml and 12 ml of 5 M NaCl was then added. This mixture was centrifuged 20 min. at 15,000 x g. The resulting pellet was resuspended in 5.7 ml of TE and 300 µl of 10% SDS and 60 µl 20 mg/ml proteinase K (Gibco BRL Products, Grand Island, NY) were added. This mixture was incubated at 37 °C for 1 hr, approximately 10 mg of lysozyme was then added and the mixture was incubated for an additional 45 min. One milliliter of 5M NaCl and 800 µl of CTAB/NaCl solution (10% w/v CTAB, 0.7 M NaCl) were then added and the mixture was incubated 10 min. at 65°C, gently agitated, then incubated and agitated for an additional 20 min. to aid in clearing of the cellular material. An equal volume of chloroform/isoamyl alcohol solution (24:1, v/v) was added, mixed gently then centrifuged. Two extractions were then performed with an equal volume of phenol/chloroform/isoamyl alcohol (50:49:1). Genomic DNA was precipitated with 0.6 volume of isopropanol. Precipitated DNA was removed with a glass rod, washed twice with 70% ethanol, dried and dissolved in 2 ml of STE (10 mM Tris-HCl pH8.0, 10 mM NaCl, 1 mM EDTA). The DNA was then quantitated by optical density at 260 nm. To perform rep-PCR analysis of *Photorhabdus* genomic DNA the following primers were used, REP1R-I; 5'-IIIICGICGICATCIGGC-3' and REP2-I; 5'-ICGICTTATCIGGCCTAC-3'. PCR was performed using the following 25µl reaction: 7.75 µl $H_2O$, 2.5 µl 10X LA buffer (PanVera Corp., Madison, WI), 16 µl dNTP mix (2.5 mM each), 1 µl of each primer at 50 pM/µl, 1 µl DMSO, 1.5 µl genomic DNA (concentrations ranged from 0.075-0.480 µg/µl) and 0.25 µl TaKaRa EX Taq (PanVera Corp., Madison, WI). The PCR amplification was performed in a Perkin Elmer DNA Thermal Cycler (Norwalk, CT) using the following conditions: 95°C/7 min. then 35 cycles of; 94°C/1 min.,44°C/1 min., 65°C/8 min., followed by 15 min. at 65°C. After cycling, the 25 µl reaction was added to 5 µl

of 6X gel loading buffer (0.25% bromophenol blue, 40% w/v sucrose in $H_2O$). A 15x20cm 1%-agarose gel was then run in TBE buffer (0.09 M Tris-borate, 0.002 M EDTA) using 8 µl of each reaction. The gel was run for approximately 16 hours at 45v. Gels were then stained in 20 µg/ml ethidium bromide for 1 hour and destained in TBE buffer for approximately 3 hours. Polaroid® photographs of the gels were then taken under UV illumination.

The presence or absence of bands at specific sizes for each strain was scored from the photographs and entered as a similarity matrix in the numerical taxonomy software program, NTSYS-pc (Exeter Software, Setauket, NY). Controls of *E. coli* strain HB101 and Xanthomonas oryzae pv. oryzae assayed at the same time produced PCR "fingerprints" corresponding to published reports (Versalovic, J., Koeuth, T. and Lupski, J.R. 1991. Nucleic Acids Res. 19, 6823-6831; Vera Cruz, C.M., Halda-Alija, L., Louws, F., Skinner, D.Z., George, M.L., Nelson, R.J., DE Bruijn, F.J., Rice, C. and Leach, J.E. 1995. Int. Rice Res. Notes, 20, 23-24.; Vera Cruz, C.M., Ardales, E.Y., Skinner, D.Z., Talag, J., Nelson, R.J., Louws, F.J., Leung, H., Mew, T.W. and Leach, J.E. 1996. Phytopathology (in press, respectively). The data from *Photorhabdus* strains were then analyzed with a series of programs within NTSYS-pc; SIMQUAL (Similarity for Qualitative data) to generate a matrix of similarity coefficients (using the Jaccard coefficient) and SAHN (Sequential, Agglomerative, Heirarchical and Nested) clustering [using the UPGMA (Unweighted Pair-Group Method with Arithmetic Averages) method] which groups related strains and can be expressed as a phenogram (Figure 5). The COPH (cophenetic values) and MXCOMP (matrix comparison) programs were used to generate a cophenetic value matrix and compare the correlation between this and the original matrix upon which the clustering was based. A resulting normalized Mantel statistic (r) was generated which is a measure of the goodness of fit for a cluster analysis (r=0.8-0.9 represents a very good fit). In our case r = 0.919. Therefore, our collection is comprised of a diverse group of easily distinguishable strains representative of the *Photorhabdus* genus.

Example 13

Insecticidal Utility of Toxin(s) Produced by Various *Photorhabdus* Strains

Initial "seed" cultures of the various *Photorhabdus* strains were produced by inoculating 175 ml of 2% Proteose Peptone #3 (PP3) (Difco Laboratories, Detroit, MI) liquid media with a primary variant subclone in a 500 ml tribaffled flask with a Delong neck, covered with a Kaput. Inoculum for each seed culture was derived from oil-overlay agar slant cultures or plate cultures. After inoculation, these flasks were incubated for 16 hrs at 28°C on a rotary shaker at 150 rpm. These seed cultures were then used as uniform inoculum sources for a given fermentation of each strain. Additionally, overlaying the post-log seed culture with sterile mineral oil, adding a sterile magnetic stir bar for future resuspension and storing the culture in the dark, at room temperature provided long-term preservation of inoculum in a toxin-competent state. The production broths were inoculated by adding 1% of the actively growing seed culture to fresh 2% PP3 media (*e.g.* 1.75 ml per 175 ml fresh media). Production of broths occurred in either 500 ml tribaffled flasks (see above), or 2800 ml baffled, convex bottom flasks (500 ml volume) covered by a silicon foam closure. Production flasks were incubated for 24-48 hrs under the above mentioned conditions. Following incubation, the broths were dispensed into sterile 1 L polyethylene bottles, spun at 2600 x g for 1 hr at 10°C and decanted from the cell and debris pellet. The liquid broth was then vacuum filtered through Whatman GF/D (2.7 µM retention) and GF/B (1.0 µM retention) glass filters to remove debris. Further broth clarification was achieved with a tangential flow microfiltration device (Pall Filtron, Northborough, MA) using a 0.5 µM open-channel filter. When necessary, additional clarification could be obtained by chilling the broth (to 4°C) and centrifuging for several hours at 2600 x g. Following these procedures, the broth was filter sterilized using a 0.2 µM nitrocellulose membrane filter. Sterile broths were then used directly for biological assay, biochemical analysis or concentrated (up to 15-fold) using a 10,000 MW cut-off, M12 ultra-filtration device (Amicon, Beverly MA) or

centrifugal concentrators (Millipore, Bedford, MA and Pall Filtron, Northborough, MA) with a 10,000 MW pore size. In the case of centrifugal concentrators, the broth was spun at 2000 x g for approximately 2 hr. The 10,000 MW permeate was added to the corresponding retentate to achieve the desired concentration of components greater than 10,000 MW. Heat inactivation of processed broth samples was acheived by heating the samples at 100°C in a sand-filled heat block for 10 minutes.

The broth(s) and toxin complex(es) from different *Photorhabdus* strains are useful for reducing populations of insects and were used in a method of inhibiting an insect population which comprises applying to a locus of the insect an effective insect inactivating amount of the active described. A demonstration of the breadth of insecticidal activity observed from broths of a selected group of *Photorhabdus* strains fermented as described above is shown in Table 19. It is possible that additional insecticidal activities could be detected with these strains through increased concentration of the broth or by employing different fermentation methods. Consistent with the activity being associated with a protein, the insecticidal activity of all strains tested was heat labile (see above).

Culture broth(s) from diverse *Photorhabdus* strains show differential insecticidal activity (mortality and/or growth inhibition, reduced adult emergence) against a number of insects. More specifically, the activity is seen against corn rootworm larvae and boll weevil larvae which are members of the insect order *Coleoptera*. Other members of the *Coleoptera* include wireworms, pollen beetles, flea beetles, seed beetles and Colorado potato beetle. Activity is also observed against aster leafhopper and corn plant hopper, which are members of the order *Homoptera*. Other members of the *Homoptera* include planthoppers, pear psylla, apple sucker, scale insects, whiteflies, spittle bugs as well as numerous host specific aphid species. The broths and purified toxin complex(es) are also active against tobacco budworm, tobacco hornworm and European corn borer which are members of the order *Lepidoptera*. Other typical members of this order are beet armyworm, cabbage looper, black cutworm, corn earworm, codling moth, clothes moth, Indian mealmoth, leaf rollers, cabbage worm, cotton bollworm, bagworm, Eastern tent

caterpillar, sod webworm and fall armyworm. Activity is also seen against fruitfly and mosquito larvae which are members of the order *Diptera*. Other members of the order *Diptera* are, pea midge, carrot fly, cabbage root fly, turnip root fly, onion fly, crane fly and house fly and various mosquito species. Activity with broth(s) and toxin complex(es) is also seen against two-spotted spider mite which is a member of the order *Acarina* which includes strawberry spider mites, broad mites, citrus red mite, European red mite, pear rust mite and tomato russet mite.

Activity against corn rootworm larvae was tested as follows. *Photorhabdus* culture broth(s) (0-15 fold concentrated, filter sterilized), 2% Proteose Peptone #3, purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer , pH 7.0 were applied directly to the surface (about 1.5 $cm^2$) of artificial diet (Rose, R. I. and McCabe, J. M. (1973). J. Econ. Entomol. 66, (398-400) in 40 µl aliquots. Toxin complex was diluted in 10 mM sodium phosphate buffer, pH 7.0. The diet plates were allowed to air-dry in a sterile flow-hood and the wells were infested with single, neonate *Diabrotica undecimpunctata howardi* (Southern corn rootworm, SCR) hatched from surface sterilized eggs. The plates were sealed, placed in a humidified growth chamber and maintained at 27°C for the appropriate period (3-5 days). Mortality and larval weight determinations were then scored. Generally, 16 insects per treatment were used in all studies. Control mortality was generally less than 5%.

Activity against boll weevil (*Anthomonas grandis*) was tested as follows. Concentrated (1-10 fold) *Photorhabdus* broths, control medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 were applied in 60 µl aliquots to the surface of 0.35 g of artificial diet (Stoneville Yellow lepidopteran diet) and allowed to dry. A single, 12-24 hr boll weevil larva was placed on the diet, and the wells were sealed and held at 25°C, 50% RH for 5 days. Mortality and larval weights were then assessed. Control mortality ranged between 0-13%.

Activity against mosquito larvae was tested as follows. The assay was conducted in a 96-well microtiter plate. Each well contained 200 µl of aqueous solution (10-fold concentrated *Photorhabdus* culture broth(s), control medium (2% Proteose

Peptone #3), 10 mM sodium phosphate buffer, toxin complex(es) @ 0.23 mg/ml or $H_2O$) and approximately 20, 1-day old larvae (*Aedes aegypti*). There were 6 wells per treatment. The results were read at 3-4 days after infestation. Control mortality was between 0-20%.

Activity against fruitflies was tested as follows. Purchased *Drosophila melanogaster* medium was prepared using 50% dry medium and a 50% liquid of either water, control medium (2% Proteose Peptone #3), 10-fold concentrated *Photorhabdus* culture broth(s), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer , pH 7.0. This was accomplished by placing 4.0 ml of dry medium in each of 3 rearing vials per treatment and adding 4.0 ml of the appropriate liquid. Ten late instar *Drosophila melanogaster* maggots were then added to each 25 ml vial. The vials were held on a laboratory bench, at room temperature, under fluorescent ceiling lights. Pupal or adult counts were made after 15 days of exposure. Adult emergence as compared to water and control medium (0-16% reduction).

Activity against aster leafhopper adults (*Macrosteles severini*) and corn planthopper nymphs (*Peregrinus maidis*) was tested with an ingestion assay designed to allow ingestion of the active without other external contact. The reservoir for the active/"food" solution is made by making 2 holes in the center of the bottom portion of a 35X10 mm Petri dish. A 2 inch Parafilm M® square is placed across the top of the dish and secured with an "O" ring. A 1 oz. plastic cup is then infested with approximately 7 hoppers and the reservoir is placed on top of the cup, Parafilm down. The test solution is then added to the reservoir through the holes. In tests using 10-fold concentrated *Photorhabdus* culture broth(s), the broth and control medium (2% Proteose Peptone #3) were dialyzed against 10 mM sodium phosphate buffer, pH 7.0 and sucrose (to 5%) was added to the resulting solution to reduce control mortality. Purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 was also tested. Mortality is reported at day 3. The assay was held in an incubator at 28°C, 70% RH with a 16/8 photoperiod. The assays were graded for mortality at 72 hours. Control mortality was less than 6%.

Activity against lepidopteran larvae was tested as follows. Concentrated (10-fold) *Photorhabdus* culture broth(s), control medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 were applied directly to the surface (~1.5 $cm^2$) of standard artificial lepidopteran diet (Stoneville Yellow diet) in 40 µl aliquots. The diet plates were allowed to air-dry in a sterile flow-hood and each well was infested with a single, neonate larva. European corn borer (*Ostrinia nubilalis*) and tobacco hornworm (*Manduca sexta*) eggs were obtained from commercial sources and hatched in-house, whereas tobacco budworm (*Heliothis virescens*) larvae were supplied internally. Following infestation with larvae, the diet plates were sealed, placed in a humidified growth chamber and maintained in the dark at 27°C for the appropriate period. Mortality and weight determinations were scored at day 5. Generally, 16 insects per treatment were used in all studies. Control mortality generally ranged from 4-12.5% for control medium and was less than 10% for phosphate buffer.

Activity against two-spotted spider mite (*Tetranychus urticae*) was determined as follows. Young squash plants were trimmed to a single cotyledon and sprayed to run-off with 10-fold concentrated broth(s), control medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0. After drying, the plants were infested with a mixed population of spider mites and held at lab temperature and humidity for 72 hr. Live mites were then counted to determine levels of control.

Table 19
Observed Insecticidal Spectrum of Broths From Different *Photorhabdus* Strains

| *Photorhabdus* Strain | Sensitive* Insect Species |
|---|---|
| WX-1 | 3**, 4, 5, 6, 7, 8 |
| WX-2 | 2, 4 |
| WX-3 | 1, 4 |
| WX-4 | 1, 4 |
| WX-5 | 4 |
| WX-6 | 4 |
| WX-7 | 3, 4, 5, 6, 7, 8 |
| WX-8 | 1, 2, 4 |
| WX-9 | 1, 2, 4 |
| WX-10 | 4 |
| WX-11 | 1, 2, 4 |
| WX-12 | 2, 4, 5, 6, 7, 8 |
| WX-14 | 1, 2, 4 |
| WX-15 | 1, 2, 4 |
| W30 | 3, 4, 5, 8 |
| NC-1 | 1, 2, 3, 4, 5, 6, 7, 8, 9 |
| WIR | 2, 3, 5, 6, 7, 8 |
| HP88 | 1, 3, 4, 5, 7, 8 |
| Hb | 3, 4, 5, 7, 8 |
| Hm | 1, 2, 3, 4, 5, 7, 8 |
| H9 | 1, 2, 3, 4, 5, 6, 7, 8 |
| W-14 | 1, 2, 3, 4, 5, 6, 7, 8, 10 |
| ATCC 43948 | 4 |
| ATCC 43949 | 4 |
| ATCC 43950 | 4 |
| ATCC 43951 | 4 |
| ATCC 43952 | 4 |

* = ≥ 25% mortality and/or growth inhibition vs. control
** = 1; Tobacco budworm, 2; European corn borer, 3; Tobacco hornworm, 4; Southern corn rootworm, 5; Boll weevil, 6; Mosquito, 7; Fruit Fly, 8; Aster Leafhopper, 9; Corn planthopper, 10; Two-spotted spider mite.

Example 14

Non W-14 *Photorhabdus* Strains:

Purification, Characterization and Activity Spectrum

Purification

The protocol, as follows, is similar to that developed for the purification of W-14 and was established based on purifying those fractions having the most activity against Southern corn root worm (SCR), as determined in bioassays (see Example 13). Typically, 4-20 L of broth that had been filtered, as described in Example 13, were received and concentrated using an Amicon spiral ultra filtration cartridge Type S1Y100 attached to an Amicon M-12 filtration device. The retentate contained native proteins consisting of molecular sizes greater than 100 kDa, whereas the flow through material contained native proteins less than 100 kDa in size. The majority of the activity against SCR was contained in the 100 kDa retentate. The retentate was then continually diafiltered with 10 mM sodium phosphate (pH = 7.0) until the filtrate reached an $A_{280}$ < 0.100. Unless otherwise stated, all procedures from this point were performed in buffer as defined by 10 mM sodium phosphate (pH 7.0). The retentate was then concentrated to a final volume of approximately 0.20 L and filtered using a 0.45 mm Nalgene™ Filterware sterile filtration unit. The filtered material was loaded at 7.5 ml/min onto a Pharmacia HR16/10 column which had been packed with PerSeptive Biosystem Poros® 50 HQ strong anion exchange matrix equilibrated in buffer using a PerSeptive Biosystem Sprint® HPLC system. After loading, the column was washed with buffer until an $A_{280}$ < 0.100 was achieved. Proteins were then eluted from the column at 2.5 ml/min using buffer with 0.4 M NaCl for 20 min for a total volume of 50 ml. The column was then washed using buffer with 1.0 M NaCl at the same flow rate for an additional 20 min (final volume = 50 ml). Proteins eluted with 0.4 M and 1.0 M NaCl were placed in separate dialysis bags (Spectra/Por® Membrane MWCO: 2,000) and allowed to dialyze overnight at 4° C in 12 L buffer. The majority of the activity against SCR was contained in the 0.4 M fraction. The 0.4 M fraction was further purified by application of 20 ml to a Pharmacia XK 26/100 column that had been prepacked with Sepharose CL4B (Pharmacia) using a flow rate

of 0.75 ml/min. Fractions were pooled based on $A_{280}$ peak profile and concentrated to a final volume of 0.75 ml using a Millipore Ultrafree®-15 centrifugal filter device Biomax-50K NMWL membrane. Protein concentrations were determined using a Biorad Protein Assay Kit with bovine gamma globulin as a standard.

Characterization

The native molecular weight of the SCR toxin complex was determined using a Pharmacia HR 16/50 that had been prepacked with Sepharose CL4B in buffer. The column was then calibrated using proteins of known molecular size thereby allowing for calculation of the toxin approximate native molecular size. As shown in Table 20, the molecular size of the toxin complex ranged from 777 kDa with strain Hb to 1,900 kDa with strain WX-14. The yield of toxin complex also varied, from strain WX-12 producing 0.8 mg/L to strain Hb, which produced 7.0 mg/L.

Proteins found in the toxin complex were examined for individual polypeptide size using SDS-PAGE analysis. Typically, 20 mg protein of the toxin complex from each strain was loaded onto a 2-15% polyacrylamide gel (Integrated Separation Systems) and electrophoresed at 20 mA in Biorad SDS-PAGE buffer. After completion of electrophoresis, the gels were stained overnight in Biorad Coomassie blue R-250 (0.2% in methanol: acetic acid: water; 40:10:40 v/v/v). Subsequently, gels were destained in methanol:acetic acid: water; 40:10:40 (v/v/v). The gels were then rinsed with water for 15 min and scanned using a Molecular Dynamics Personal Laser Densitometer®. Lanes were quantitated and molecular sizes were calculated as compared to Biorad high molecular weight standards, which ranged from 200-45 kDa.

Sizes of the individual polypeptides comprising the SCR toxin complex from each strain are listed in Table 21. The sizes of the individual polypeptides ranged from 230 kDa with strain WX-1 to a size of 16 kDa, as seen with strain WX-7. Every strain, with the exception of strain Hb, had polypeptides comprising the toxin complex that were in the 160-230 kDa range, the 100-160 kDa range, and the 50-80 kDa range. These data indicate that the toxin complex may vary in peptide composition and components from strain to strain, however, in all cases the

toxin attributable appears to consist of a large oligomeric protein complex.

Table 20

Characterization of a Toxin Complex From Non W-14 *Photorhabdus* Strains

| Strain | Approx. Native Molecular Wt.[a] | Yield Active Fraction (mg/L)[b] |
|---|---|---|
| H9 | 972,000 | 1.8 |
| Hb | 777,000 | 7.0 |
| Hm | 1,400,000 | 1.1 |
| HP88 | 813,000 | 2.5 |
| NC1 | 1,092,000 | 3.3 |
| WIR | 979,000 | 1.0 |
| WX-1 | 973,000 | 0.8 |
| WX-2 | 951,000 | 2.2 |
| WX-7 | 1,000,000 | 1.5 |
| WX-12 | 898,000 | 0.4 |
| WX-14 | 1,900,000 | 1.9 |
| W-14 | 860,000 | 7.5 |

a Native molecular weight determined using a Pharmacia HR 16/50 column packed with Sepharose CL4B
b Amount of toxin complex recovered from culture broth.

Activity Spectrum

As shown in Table 21, the toxin complexes purified from strains Hm and H9 were tested for activity against a variety of insects, with the toxin complex from strain W-14 for comparison. The assays were performed as described in Example 13. The toxin complex from all three strains exhibited activity against tobacco bud worm, European corn borer, Southern corn root worm, and aster leafhopper. Furthermore, the toxin complex from strains Hm and W-14 also exhibited activity against two-spotted spider mite. In addition, the toxin complex from W-14 exhibited activity against mosquito larvae. These data indicate that the toxin complex, while having similarities in activities between certain orders of insects, can also exhibit differential activities against other orders of insects.

Table 21

The Approximate Sizes (in kDa) of Peptides in a Purified Toxin Complex From Non W-14 *Photorhabdus*

| H9 | Hb | Hm | HP 88 | NC-1 | WIR | WX-1 | WX-2 | WX-7 | WX-12 | WX-14 | W-11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 180 | 150 | 170 | 170 | 180 | 170 | 230 | 200 | 200 | 180 | 210 | 190 |
| 170 | 140 | 140 | 160 | 170 | 160 | 190 | 170 | 180 | 160 | 180 | 180 |
| 160 | 139 | 100 | 140 | 140 | 120 | 170 | 150 | 110 | 140 | 160 | 170 |
| 140 | 130 | 81 | 130 | 110 | 110 | 160 | 120 | 87 | 139 | 120 | 160 |
| 120 | 120 | 72 | 129 | 44 | 89 | 110 | 110 | 75 | 130 | 110 | 150 |
| 98 | 100 | 68 | 110 | 16 | 79 | 98 | 82 | 43 | 110 | 100 | 130 |
| 87 | 98 | 49 | 100 | | 74 | 76 | 64 | 33 | 92 | 95 | 120 |
| 84 | 88 | 46 | 86 | | 62 | 58 | 37 | 28 | 87 | 80 | 110 |
| 79 | 81 | 30 | 81 | | 51 | 53 | 30 | 26 | 80 | 69 | 93 |
| 72 | 75 | 22 | 77 | | 40 | 41 | | 23 | 73 | 49 | 90 |
| 68 | 69 | 20 | 73 | | 39 | 35 | | 22 | 59 | 41 | 77 |
| 60 | 60 | 19 | 60 | | 37 | 31 | | 21 | 56 | 33 | 69 |
| 57 | 57 | | 58 | | 33 | 28 | | 19 | 51 | | 65 |
| 52 | 54 | | 45 | | 30 | 24 | | 18 | 37 | | 63 |
| 46 | 49 | | 39 | | 28 | 22 | | 16 | 33 | | 60 |
| 40 | 44 | | 35 | | 27 | | | | 32 | | 51 |
| 37 | 39 | | | | 25 | | | | 26 | | 46 |
| | 37 | | | | 23 | | | | | | 40 |
| | 35 | | | | | | | | | | 39 |
| | | | | | | | | | | | 29 |

Table 22

Observed Insecticidal Spectrum of a Purified Toxin Complex from *Photorhabdus* Strains

| *Photorhabdus* Strain | Sensitive* Insect Species |
|---|---|
| Hm Toxin Complex | 1**, 2, 3, 5, 6, 7, 8 |
| H9 Toxin Complex | 1, 2, 3, 6, 7, 8 |
| W-14 Toxin Complex | 1, 2, 3, 4, 5, 6, 7, 8 |

* = > 25% mortality or growth inhibition
* = > 25% mortality or growth inhibition

** = 1; Tobacco bud worm, 2; European corn borer, 3; Southern corn root worm, 4; Mosquito, 5; Two-spotted spider mite, 6; Aster Leafhopper, 7; Fruit Fly, 8; Boll Weevil

## Example 15
## Sub-Fractionation of *Photorhabdus* Protein Toxin Complex

The *Photorhabdus* protein toxin complex was isolated as described in Example 14. Next, about 10 mg toxin was applied to a MonoQ 5/5 column equilibrated with 20 mM Tris-HCl, pH 7.0 at a flow rate of 1ml/min. The column was washed with 20 mM Tris-HCl, pH 7.0 until the optical density at 280 nm returned to baseline absorbance. The proteins bound to the column were eluted with a linear gradient of 0 to 1.0 M NaCl in 20 mM Tris-HCl, pH 7.0 at 1 ml/min for 30 min. One ml fractions were collected and subjected to Southern corn rootworm (SCR) bioassay (see Example 13). Peaks of activity were determined by a series of dilutions of each fraction in SCR bioassays. Two activity peaks against SCR were observed and were named A (eluted at about 0.2-0.3 M NaCl) and B (eluted at 0.3-0.4 M NaCl). Activity peaks A and B were pooled separately and both peaks were further purified using a 3-step procedure described below.

Solid $(NH_4)_2SO_4$ was added to the above protein fraction to a final concentration of 1.7 M. Proteins were then applied to a phenyl-Superose 5/5 column equilibrated with 1.7 M $(NH_4)_2SO_4$ in 50 mM potassium phosphate buffer, pH 7 at 1 ml/min. Proteins bound to the column were eluted with a linear gradient of 1.7 M $(NH_4)_2SO_4$, 0% ethylene glycol, 50 mM potassium phosphate, pH 7.0 to 25% ethylene glycol, 25 mM potassium phosphate, pH 7.0 (no $(NH_4)_2SO_4$) at 0.5 ml/min. Fractions were dialyzed overnight

against 10 [redacted] sodium phosphate buffer, pH [redacted]. Activities in each fraction against SCR were determined by bioassay.

The fractions with the highest activity were pooled and applied to a MonoQ 5/5 column which was equilibrated with 20 mM Tris-HCl, pH 7.0 at 1 ml/min. The proteins bound to the column were eluted at 1 ml/min by a linear gradient of 0 to 1M NaCl in 20 mM Tris-HCl, pH 7.0.

For the final step of purification, the most active fractions above (determined by SCR bioassay) were pooled and subjected to a second phenyl-Superose 5/5/ column. Solid $(NH_4)_2SO_4$ was added to a final concentration of 1.7 M. The solution was then loaded onto the column equilibrated with 1.7 M $(NH_4)_2SO_4$ in 50 mM potassium phosphate buffer, pH 7 at 1ml/min. Proteins bound to the column were eluted with a linear gradient of 1.7 M $(NH_4)_2SO_4$, 50 mM potassium phosphate, pH 7.0 to 10 mM potassium phosphate, pH 7.0 at 0.5 ml/min. Fractions were dialyzed overnight against 10 mM sodium phosphate buffer, pH 7.0. Activities in each fraction against SCR were determined by bioassay.

The final purified protein by the above 3-step procedure from peak A was named toxin A and the final purified protein from peak B was named toxin B.

Characterization and Amino Acid Sequencing of Toxin A and Toxin B

In SDS-PAGE, both toxin A and toxin B contained two major (> 90% of total Commassie stained protein) peptides: 192 kDa (named A1 and B1, respectively) and 58 kDa (named A2 and B2, respectively). Both toxin A and toxin B revealed only one major band in native PAGE, indicating A1 and A2 were subunits of one protein complex, and B1 and B2 were subunits of one protein complex. Further, the native molecular weight of both toxin A and toxin B were determined to be 860 kDa by gel filtration chromatography. The relative molar concentrations of A1 to A2 was judged to be a 1 to 1 equivalence as determined by densiometric analysis of SDS-PAGE gels. Similarly, B1 and B2 peptides were present at the same molar concentration.

Toxin A and toxin B were electrophoresed in 10% SDS-PAGE and transblotted to PVDF membranes. Blots were sent for amino acid analysis and N-terminal amino acid sequencing at Harvard MicroChem and Cambridge ProChem, respectively. The N-terminal

amino sequen f B1 was determined to be i[redacted]tical to SEQ ID NO:1, the $TcbA_{ii}$ region of the *tcbA* gene (SEQ ID NO:12, position 87 to 99). A unique N-terminal sequence was obtained for peptide B2 (SEQ ID NO:40). The N-terminal amino acid sequence of peptide B2 was identical to the $TcbA_{iii}$ region of the derived amino acid sequence for the *tcbA* gene (SEQ ID NO:12, position 1935 to 1945). Therefore, the B toxin contained predominantly two peptides, $TcbA_{ii}$ and $TcbA_{iii}$, that were observed to be derived from the same gene product, TcbA.

The N-terminal sequence of A2 (SEQ ID NO:41) was unique in comparison to the $TcbA_{iii}$ peptide and other peptides. The A2 peptide was denoted $TcdA_{iii}$ (see Example 17). SEQ ID NO:6 was determined to be a mixture of amino acid sequences SEQ ID NO:40 and 41.

Peptides A1 and A2 were further subjected to internal amino acid sequencing. For internal amino acid sequencing, 10 µg of toxin A was electrophoresized in 10% SDS-PAGE and transblotted to PVDF membrane. After the blot was stained with amido black, peptides A1 and A2, denoted $TcdA_{ii}$ and $TcdA_{iii}$, respectively, were excised from the blot and sent to Harvard MicroChem and Cambridge ProChem. Peptides were subjected to trypsin digestion followed by HPLC chromatography to separate individual peptides. N-terminal amino acid analysis was performed on selected tryptic peptide fragments. Two internal amino acid sequences of peptide A1 ($TcdA_{ii}$-PK71, SEQ ID NO:38 and $TcdA_{ii}$-PK44, SEQ ID NO:39) were found to have significant homologies with deduced amino acid sequences of the $TcbA_{ii}$ region of the *tcbA* gene (SEQ ID NO:12). Similarly, the N-terminal sequence (SEQ ID NO:41) and two internal sequences of peptides A2 ($TcdA_{iii}$-PK57, SEQ ID NO:42 and $TcdA_{iii}$-PK20, SEQ ID NO.43) also showed significant homology with deduced amino acid sequences of $TcbA_{iii}$ region of the *tcbA* gene (SEQ ID NO:12).

In summary of above results, the toxin complex has at least two active protein toxin complexes against SCR; toxin A and toxin B. Toxin A and toxin B are similar in their native and subunits molecular weight, however, their peptide compositions are different. Toxin A contained peptides $TcdA_{ii}$ and $TcdA_{iii}$ as the major peptides and the toxin B contains $TcbA_{ii}$ and $TcbA_{iii}$ as the major peptides.

## Example 16

## Cleavage and Activation of TcbA Peptide

In the toxin B complex, peptide $TcbA_{ii}$ and $TcbA_{iii}$ originate from the single gene product TcbA (Example 15). The processing of TcbA peptide to $TcbA_{ii}$ and $TcbA_{iii}$ is presumably by the action of *Photorhabdus* protease(s), and most likely, the metalloproteases described in Example 10. In some cases, it was noted that when *Photorhabdus* W-14 broth was processed, TcbA peptide was present in toxin B complex as a major component, in addition to peptides $TcbA_{ii}$ and $TcbA_{iii}$. Identical procedures, described for the purification of toxin B complex (Example 15), were used to enrich peptide TcbA from toxin complex fraction of W-14 broth. The final purified material was analyzed in a 4-20% gradient SDS-PAGE and major peptides were quantified by densitometry. It was determined that TcbA, $TcbA_{ii}$ and $TcbA_{iii}$ comprised 58%, 36%, and 6%, respectively, of total protein. The identities of these peptides were confirmed by their respective molecular sizes in SDS-PAGE and Western blot analysis using monospecific antibodies. The native molecular weight of this fraction was determined to be 860 kDa.

The cleavage of TcbA was evaluated by treating the above purified material with purified 38 kDa and 58 kDa W-14 *Photorhabdus* metalloproteases (Example 10), and Trypsin as a control enzyme (Sigma, MO). The standard reaction consisted 17.5 µg the above purified fraction, 1.5 unit protease, and 0.1 M Tris buffer, pH 8.0 in a total volume of 100 µl. For the control reaction, protease was omitted. The reaction mixtures were incubated at 37 °C for 90 min. At the end of the reaction, 20 µl was taken and boiled with SDS-PAGE sample buffer immediately for electrophoresis analysis in a 4-20% gradient SDS-PAGE. It was determined from SDS-PAGE that in both 38 kDa and 58 kDa protease treatments, the amount of peptides $TcbA_{ii}$ and $TcbA_{iii}$ increased about 3-fold while the amount of TcbA peptide decreased proportionally (Table 23). The relative reduction and augmentation of selected peptides was confirmed by Western blot analyses. Furthermore, gel filtration of the cleaved material revealed that the native molecular size of the complex remained the same. Upon trypsin treatment, peptides TcbA and $TcbA_{ii}$ were

nonspecifically digested into small peptides. This indicated that 38 kDa and 58 kDa *Photorhabdus* proteases can specifically process peptide TcbA into peptides $TcbA_{ii}$ and $TcbA_{iii}$. Protease treated and untreated control of the remaining 80 ul reaction mixture were serial diluted with 10 mM sodium phosphate buffer, pH 7.0 and analyzed by SCR bioassay. By comparing activity in several dilution, it was determined that the 38 kDa protease treatment increased SCR insecticidal activity approximately 3 to 4 fold. The growth inhibition of remaining insects in the protease treatment was also more severe than control (Table 23).

Table 23

Conversion and activation of peptide TcbA into peptides $TcbA_{ii}$ and $TcbA_{iii}$ by protease treatment.

| | Control | 38 kDa protease treatment |
|---|---|---|
| S0 (% of total protein) | 58 | 18 |
| S1 (% of total protein) | 36 | 64 |
| S9 (% of total protein) | 6 | 18 |
| LD50 (µg protein) | 2.1 | 0.52 |
| SCR Weight (mg/insect)* | 0.2 | 0.1 |

*: an indication of growth inhibition by measuring the average weight of live insect after 5 days on diet in the assay.

## Example 17

## Screening of the library for a gene encoding the $TcdA_{ii}$ Peptide

The cloning and characterization of a gene encoding the $TcdA_{ii}$ peptide, described as SEQ ID NO:17 (internal peptide $TcdA_{ii}$-PT111 N-terminal sequence) and SEQ ID NO:18 (internal peptide $TcdA_{ii}$-PT79 N-terminal sequence) was completed. Two pools of degenerate oligonucleotides, designed to encode the amino acid sequences of SEQ ID NO:17 (Table 24) and SEQ ID NO:18 (Table 25), and the reverse complements of those sequences, were synthesized as described in Example 8. The DNA sequence of the oligonucleotides is given below:

Table 24

Degenerate Oligonucleotide for SEQ ID NO:17

| P2-PT111 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Amino Acid | Ala | Phe | Asn | Ile | Asp | Asp | Val | Ser |
| Codons | 5' GCN | TT(T/C) | AA(T/C) | AT(T/C/A) | GA(T/C) | GA(T/C) | GTN 3' | |
| P2.3.6.CB | 5' GC(A/C/G/T) | TT(T/C) | AAT | ATT | GAT | GAT | GT 3' | |
| P2.3.5 | 5' GC(A/C/G/T) | TT(T/C) | AA(T/C) | AT(T/C/A) | GA(T/C) | GA(T/C) | GT 3' | |
| P2.3.5R | 5' AC | (G/A)TC | (G/A)TC | (T/G/A)AT | (G/A)TT | (G/A)AA | (A/C/G/T)GC 3' | |
| P2.3.5RI | 5' ACI | TCI | TCI | ATI | TTI | AAI | GC 3' | |
| P2.3R.CB | 5' CAG | (A/G)CT | (A/C)AC | ATC | ATC | AAT | ATT | AAA 3' |

Table 25

Degenerate Oligonucleotide for SEQ ID NO:18

| P2-PT79 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino Acid | Phe | Ile | Val | Tyr | Thr | Ser | Leu | Gly | Val | Asn | Pro | Asn | Asn |
| Codons* | 5' TTY | ATH | GTN | TAY | ACN | 6 | 6 | GGN | GTN | AAY | CCN | AAY | AAY 3' |
| P2.79.2 | 5' TTY | ATY | GTK | TAT | ACY | TCI | YTR | GGY | GTK | AAT | CCR | AAT | AAT 3' |
| P2.79.3 | 5' TTT | ATT | GTK | TAT | ACY | AGY | YTR | GGY | GTK | AAT | CCR | AAT | AAT 3' |
| P2.79.R.1 | 5' ATT | ATT | YGG | ATT | MAC | RCC | YAR | RCT | RGT | ATA | MAC | AAT | AAA 3' |
| P2.79R.CB | 5' ATT | ATT | YGG | ATT | MAC | ACC | CAG | RCT | GGT | ATA | MAC | AAT | AAA 3' |

* According to IUPAC-IUB codes for nucleotides, Y = C or T, H = A, C or T, N = A, C, G or T, K = G or T, R = A or G, and M = A or C

Polymerase Chain Reactions (PCR) were performed essentially as described in Example 8, using as forward primers P2.3.6.CB or P2.3.5, and as reverse primers P2.79.R.1 or P2.79R.CB, in all forward/reverse combinations, using *Photorhabdus* W-14 genomic DNA as template. In another set of reactions, primers P2.79.2 or P2.79.3 were used as forward primers, and P2.3.5R, P2.3.5RI, and P2.3R.CB were used as reverse primers in all forward/reverse combinations. Only in the reactions containing P2.3.6.CB as the forward primers combined with P2.79.R.1 or P2.79R.CB as the reverse primers was a non-artifactual amplified product seen, of estimated size (mobility on agarose gels) of 2500 base pairs. The order of the primers used to obtain this amplification product indicates that the peptide fragment $TcdA_{ii}$-PT111 lies amino-proximal to the peptide fragment $TcdA_{ii}$-PT79.

The 2500 bp PCR products were ligated to the plasmid vector pCR™II (Invitrogen, San Diego, CA) according to the supplier's instructions, and the DNA sequences across the ends of the insert fragments of two isolates (HS24 and HS27) were determined using the supplier's recommended primers and the sequencing methods described previously. The sequence of both isolates was the same. New primers were synthesized based on the determined sequence, and used to prime additional sequencing reactions to obtain a total of 2557 bases of the insert [SEQ ID NO:36]. Translation of the partial peptide encoded by SEQ ID No: 36 yields the 845 amino acid sequence disclosed as SEQ ID NO:37. Protein homology analysis of this portion of the $TcdA_{ii}$ peptide fragment reveals substantial amino acid homology (68% similarity; 53% identity) to residues 542 to 1390 of protein TcbA [SEQ ID NO:12]. It is therefore apparent that the gene represented in part by SEQ ID NO:36 produces a protein of similar, but not identical, amino acid sequence as the TcbA protein, and which likely has similar, but not identical biological activity as the TcbA protein.

In yet another instance, a gene encoding the peptides $TcdA_{ii}$-PK44 and the $TcdA_{iii}$ 58 kDa N-terminal peptide, described as SEQ ID NO:9 (internal peptide $TcdA_{ii}$-PK44 sequence), and SEQ ID NO:41($TcdA_{iii}$ 58 kDa N-terminal peptide sequence) was isolated. Two pools of degenerate oligonucleotides, designed to encode the amino acid sequences described as SEQ ID NO:39 (Table 27) and SEQ

ID NO:41 (Table 26), and the reverse complements of those sequences, were synthesized as described in Example 8, and their DNA sequences.

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Table 26

Degenerate Oligonucleotide for SEQ ID NO:41

| Codon # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino Acid | Leu | Arg | Ser | Ala | Asn | Thr | Leu | Thr | Asp | Leu | Phe | Leu | Pro | Glu |
| A2.1 | 5' YTR | CGY | AGY | GCI | AAT | ACY | YTR | ACY | GAT | YTR | TTT | YTR | CCR | CA 3' |
| A2.2 | | | | GCI | AAT | ACI | YTR | ACI | GAY | YTR | TTY | YTR | CCI | CA 3' |
| A2.3.R | | 5' TG | YCG | YAR | AAA | YAR | RTC | RGT | YAR | RGT | RTT | IGC | RCT | RCG 3' |
| A2.4.R | | | | 5' TG | IGG | CAG | AAA | CAG | RTC | IGT | CAG | IGT | ATT | IGC 3' |

Table 27

Degenerate Oligonucleotide for SEQ ID NO:39

| Amino Acid # | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Codon # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| Amino Acid | Gly | Pro | Val | Glu | Ile | Asn | Thr | Ala | Ile | |
| A1.44.1 | 5' GGY | CCR | GTK | GAA | ATT | AAT | ACC | GCI | AT 3' | |
| A1.44.1R | 5' ATI | GCG | GTA | TTA | ATT | TCM | ACY | GGR | CC 3' | |
| A1.44.2 | 5' GGI | CCI | GTI | GAR | ATY | AAY | ACI | GCI | AT 3' | |
| A1.44.2R | 5' ATI | GCI | GTR | TTR | ATY | TCI | ACI | GGI | CC 3' | |

Polymerase Chain Reactions (PCR) were performed essentially as described in Example 8, using as forward primers A1.44.1 or A1.44.2, and reverse primers A2.3R or A2.4R, in all forward/reverse combinations, using *Photorhabdus* W-14 genomic DNA as template. In another set of reactions, primers A2.1 or A2.2 were used as forward primers, and A1.44.1R, and A1.44.2R were used as reverse primers in all forward/reverse combinations. Only in the reactions containing A1.44.1 or A1.44.2 as the forward primers combined with A2.3R as the reverse primer was a non-artifactual amplified product seen, of estimated size (mobility on agarose gels) of 1400 base pairs. The order of the primers used to obtain this amplification product indicates that the peptide fragment $TcdA_{ii}$-PK44 lies amino-proximal to the 58 kDa peptide fragment of $TcdA_{iii}$.

The 1400 bp PCR products were ligated to the plasmid vector pCR™II according to the supplier's instructions. The DNA sequences across the ends of the insert fragments of four isolates were determined using primers similar in sequence to the supplier's recommended primers and using sequencing methods described previously. The nucleic acid sequence of all isolates differed as expected in the regions corresponding to the degenerate primer sequences, but the amino acid sequences deduced from these data were the same as the actual amino acid sequences for the peptides determined previously, (SEQ ID NOS:41 and 39).

Screening of the W-14 genomic cosmid library as described in Example 8 with a radiolabeled probe comprised of the DNA prepared above (SEQ ID NO:36) identified five hybridizing cosmid isolates, namely 17D9, 20B10, 21D2, 27B10, and 26D1. These cosmids were distinct from those previously identified with probes corresponding to the genes described as SEQ ID NO:11 or SEQ ID NO:25. Restriction enzyme analysis and DNA blot hybridizations identified three EcoR I fragments, of approximate sizes 3.7, 3.7, and 1.1 kbp, that span the region comprising the DNA of SEQ ID NO:36. Screening of the W-14 genomic cosmid library using as probe the radiolabeled 1.4 kbp DNA fragment prepared in this example identified the same five cosmids (17D9, 20B10, 21D2, 27B10, and 26D1). DNA blot hybridization to EcoR I-digested cosmid DNAs also showed hybridization to the same subset

of EcoR I fragments as seen with the 2.5 kb TcdAii gene probe, indicating that both fragments are encoded on the genomic DNA.

DNA sequence determination of the cloned EcoR I fragments revealed an uninterrupted reading frame of 7551 base pairs (SEQ ID NO:46), encoding a 282.9 kDa protein of 2516 amino acids (SEQ ID NO:47). Analysis of the amino acid sequence of this protein revealed all expected internal fragments of peptides TcdAii (SEQ ID NOS:17, 18, 37, 38 and 39) and the TcdAiii peptide N-terminus (SEQ ID NO:41) and all TcdAiii internal peptides (SEQ ID NOS:42 and 43). The peptides isolated and identified as TcdAii and TcdAiii are each products of the open reading frame, denoted *tcdA*, disclosed as SEQ ID NO:46. Further, SEQ ID NO:47 shows, starting at position 89, the sequence disclosed as SEQ ID NO:13, which is the N-terminal sequence of a peptide of size approximately 201 kDa, indicating that the initial protein produced from SEQ ID No: 46 is processed in a manner similar to that previously disclosed for SEQ ID NO:12. In addition, the protein is further cleaved to generate a product of size 209.2 kDa, encoded by SEQ ID NO:48 and disclosed as SEQ ID NO:49 (TcdAii peptide), and a product of size 63.6 kDa, encoded by SEQ ID NO:50 and disclosed as SEQ ID NO:51 (TcdAiii peptide). Thus, it is thought that the insecticidal activity identified as toxin A (Example 15) derived from the products of SEQ ID NO:46, as exemplified by the full-length protein of 282.9 kDa disclosed as SEQ ID NO:47, is processed to produce the peptides disclosed as SEQ ID NOS:49 and 51. It is thought that the insecticidal activity identified as toxin B (Example 15) derives from the products of SEQ ID NO:11, as exemplified by the 280.6 kDa protein disclosed as SEQ ID NO:12. This protein is proteolytically processed to yield the 207.6 kDa peptide disclosed as SEQ ID NO:53, which is encoded by SEQ ID NO:52, and the 62.9 kDa peptide having N-terminal sequence disclosed as SEQ ID NO:40, and further disclosed as SEQ ID NO:55, which is encoded by SEQ ID NO:54.

Amino acid sequence comparisons between the proteins disclosed as SEQ ID NO:12 and SEQ ID NO:47 reveal that they have 69% similarity and 54% identity. This high degree of evolutionary relationship is not uniform throughout the entire amino acid sequence of these peptides, but is higher towards the carboxy-terminal end of the proteins, since the peptides

disclosed as SEQ ID NO:51 (derived from SEQ ID NO:47) and SEQ ID NO:55 (derived from SEQ ID NO:12) have 76% similarity and 64% identity.

## Example 18

## Control of European Cornborer-Induced Leaf Damage on Maize Plants by Spray Application of *Photorhabdus* (Strain W-14) Broth

The ability of *Photorhabdus* toxin(s) to reduce plant damage caused by insect larvae was demonstrated by measuring leaf damage caused by European corn borer (*Ostrinia nubilalis*) infested onto maize plants treated with *Photorhabdus* broth. Fermentation broth from *Photorhabdus* strain W-14 was produced and concentrated approximately 10-fold using ultrafiltration (10,000 MW pore-size) as described in Example 13. The resulting concentrated broth was then filter sterilized using 0.2 micron nitrocellulose membrane filters. A similarly prepared sample of uninoculated 2% proteose peptone #3 was used for control purposes. Maize plants (a DowElanco proprietary inbred line) were grown from seed to vegetative stage 7 or 8 in pots containing a soilless mixture in a greenhouse (27°C day; 22°C night, about 50%RH, 14 hr day-length, watered/fertilized as needed). The test plants were arranged in a randomized complete block design (3 reps/treatment, 6 plants/treatment) in a greenhouse with temperature about 22°C day; 18°C night, no artificial light and with partial shading, about 50%RH and watered/fertilized as needed. Treatments (uninoculated media and concentrated *Photorhabdus* broth) were applied with a syringe sprayer, 2.0 mls applied from directly (about 6 inches) over the whorl and 2.0 additional mls applied in a circular motion from approximately one foot above the whorl. In addition, one group of plants received no treatment. After the treatments had dried (approximately 30 minutes), twelve neonate European corn borer larvae (eggs obtained from commercial sources and hatched in-house) were applied directly to the whorl. After one week, the plants were scored for damage to the leaves using a modified Guthrie Scale (Koziel, M. G., Beland, G. L., Bowman, C., Carozzi, N. B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M. Z., Merlin, E., Rhodes, R.,

Warren, G. W., Wright, M. and Evola, S. V. (1993). Bio/Technology, 11, 194-195.) and the scores were compared statistically [T-test (LSD) $p<0.05$ and Tukey's Studentized Range (HSD) Test $p<0.1$]. The results are shown in Table 28. For reference, a score of 1 represents no damage, a score of 2 represents fine "window pane" damage on the unfurled leaf with no pinhole penetration and a score of 5 represents leaf penetration with elongated lesions and/or mid rib feeding evident on more than three leaves (lesions < 1 inch). These data indicate that broth or other protein containing fractions may confer protection against specific insect pests when delivered in a sprayable formulation or when the gene or derivative thereof, encoding the protein or part thereof, is delivered via a transgenic plant or microbe.

Table 28

Effect of *Photorhabdus* Culture Broth on European Corn Borer-Induced Leaf Damage on Maize

| **Treatment** | **Average Guthrie Score** |
|---|---|
| No Treatment | 5.02[a] |
| Uninoculated medium | 5.15[a] |
| *Photorhabdus* Broth | 2.24[b] |

Means with different letters are statistically different ($p<0.05$ or $p<0.1$).

## Example 19

## Genetic Engineering of Genes for Expression in *E. coli*

### Summary of constructions

A series of plasmids were constructed to express the *tcbA* gene of *Photorhabdus* W-14 in *Escherichia coli*. A list of the plasmids is shown in Table 29. A brief description of each construction follows as well as a summary of the *E. coli* expression data obtained.

Table 29

Expression plasmids for the *tcbA* gene.

| Plasmid | Gene | Vector/Selection | Compartment |
|---|---|---|---|
| | | | |
| pDAB634 | *tcbA* | pBC/Chl | Intracellular |
| pAcGP67B/ *tcbA* | *tcbA* | pAcGP67B/Amp | Baculovirus, secreted |
| pDAB635 | *tcbA* | pET27b/Kan | Periplasm |
| pET15-*tcbA* | *tcbA* | pET15-*tcbA* | Intracellular |

Abbreviations: Kan=kanamycin, Chl=chloramphenicol, Amp=ampicillin

Construction of pDAB634

In Example 9, a large EcoR I fragment which hybridizes to the $TcbA_{ii}$ probe is described. This fragment was subcloned into pBC (Stratagene, La Jolla CA). Sequence analysis indicates that this fragment is 8816 base pairs. The fragment encodes the *tcbA* gene with the initiating ATG at position 571 and the terminating TAA at position 8086. The fragment therefore carries 570 base pairs of *Photorhabdus* DNA upstream of the ATG and 730 base pairs downstream of the TAA.

Construction of Plasmid pAcGP67B/*tcbA*

The *tcbA* gene was PCR amplified using the following primers; 5' primer (S1Ac51) 5' TTT AAA CCA TGG GAA ACT CAT TAT CAA GCA CTA TC 3' and 3' primer (S1Ac31) 5' TTT AAA GCG GCC GCT TAA CGG ATG GTA TAA CGA ATA TG 3'. PCR was performed using a TaKaRa LA PCR kit from PanVera (Madison, Wisconsin) in the following reaction: 57.5 ml water, 10 ml 10X LA buffer, 16 ml dNTPs (2.5 mM each stock solution), 20 ml each primer at 10 pmoles/ml, 300 ng of the plasmid pDAB634 containing the W-14 *tcbA* gene and one ml of TaKaRa LA Taq polymerase. The cycling conditions were 98°C/20 sec, 68°C/5 min, 72°C/10 min for 30 cycles. A PCR product of the expected about 7526bp was isolated in a 0.8% agarose gel in TBE (100 mM Tris, 90 mM boric acid, 1 mM EDTA) buffer and purified using a Qiaex II kit from Qiagen (Chatsworth, California). The purified *tcbA* gene was digested with Nco I and Not I and ligated into the baculovirus transfer vector pAcGP67B (PharMingen (San Diego, California)) and transformed into DH5α *E. coli*. The tcbA gene was then cut from pAcGP67B and transferred to pET27b to create plasmid pDAB635. A missense mutation in the tcbA gene was repaired in pDAB635.

The reported *tcbA* gene contains two changes from the sequence shown in Sequence ID NO:11; an A->G at 212 changing an asparagine 71 to serine 71 and a G->A at 229 changing an alanine 77 to threonine 77. These changes are both upstream of the proposed $TcbA_{ii}$ N-terminus.

Construction of pET15-*tcbA*

The *tcbA* coding region of pDAB635 was transferred to vector pET15b. This was accomplished using shotgun ligations, the DNAs were cut with restriction enzymes Nco I and Xho I. The resulting recombinant is called pET15-*tcbA*.

Expression of TcbA in *E. coli* from plasmid pET15-*tcbA*

Expression of *tcbA* in *E. coli* was obtained by modification of the methods previously described by Studier *et al.* (Studier, F.W., Rosenberg, A., Dunn, J., and Dubendorff, J., (1990) Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol., 185: 60-89.). Competent *E. coli* cells strain BL21(DE3) were transformed with plasmid pET15-*tcbA* and plated on LB agar containing 100 µg/ml ampicillin and 40 mM glucose. The transformed cells were plated to a density of several hundred isolated colonies/plate. Following overnight incubation at 37°C the cells were scraped from the plates and suspended in LB broth containing 100 µg /ml ampicillin. Typical culture volumes were from 200-500 ml. At time zero, culture densities (OD600) were from 0.05-0.15 depending on the experiment. Cultures were shaken at one of three temperatures (22°C, 30°C or 37°C) until a density of 0.15-0.5 was obtained at which time they were induced with 1 mM isopropylthio-β-galactoside (IPTG). Cultures were incubated at the designated temperature for 4-5 hours and then were transferred to 4°C until processing (12-72 hours).

Purification and characterization of TcbA expressed in *E.coli* from Plasmid pET15-*tcbA*.

*E. coli* cultures expressing TcbA peptides were processed as follows. Cells were harvested by centrifugation at 17,000 x G and the media was decanted and saved in a separate container.

The media was concentrated about 8x using the M12 (Amicon, Beverly MA) filtration system and a 100 kD molecular mass cut-off filter. The concentrated media was loaded onto an anion exchange

column and the bound proteins were eluted with 1.0 M NaCl. The 1.0 M NaCl elution peak was found to cause mortality against Southern corn rootworm (SCR) larvae Table 30). The 1.0 M NaCl fraction was dialyzed against 10 mM sodium phosphate buffer pH 7.0, concentrated, and subjected to gel filtration on Sepharose CL-4B (Pharmacia, Piscataway, New Jersey). The region of the CL-4B elution profile corresponding to calculated molecular weight (about 900 kDa) as the native W-14 toxin complex was collected, concentrated and bioassayed against larvae. The collected 900 kDa fraction was found to have insecticidal activity (see Table 30 below), with symptomology similar to that caused by native W-14 toxin complex. This fraction was subjected to Proteinase K and heat treatment, the activity in both cases was either eliminated or reduced, providing evidence that the activity is proteinaceous in nature. In addition, the active fraction tested immunologically positive for the TcbA and $TcbA_{iii}$ peptides in immunoblot analysis when tested with an anti-$TcbA_{iii}$ monoclonal antibody (Table 30).

Table 30

Results of Immunoblot and SCR Bioassays.

| Fraction | SCR Activity | | Immunoblot | Native Size |
|---|---|---|---|---|
| | % Mortality | % Growth Inhibit. | Peptides Detected | [CL-4B Estimated Size] |
| TcbA Media 1.0 M Ion Exchange | +++ | +++ | TcbA | |
| TcbA Media CL-4B | +++ | +++ | TcbA, $TcbA_{iii}$ | ~900 kDa |
| TcbA Media CL-4B + Proteinase K | ++ | +++ | NT | |
| TcbA Media CL-4B + heat treatment | - | - | NT | |
| | | | | |
| | | | | |
| TcbA Cell Sup CL-4B | - | +++ | NT | ~900 kD |
| | | | | |

PK = Proteinase K treatment 2 hours; Heat treatment = 100°C for 10 minutes; ND = None Detected; NT = Not Tested. Scoring system for mortality and growth inhibition as compared to control samples; 5-24%="+", 25-49%="++", 50-100%="+++".

The cell pellet was resuspended in 10 mM sodium phosphate buffer, pH=7.0, and lysed by passage through a Bio-Neb™ cell nebulizer (Glas-Col Inc., Terra Haute, IN). The pellets were

treated with DNase to remove DNA and centrifuged at 17,000 x g to separate the cell pellet from the cell supernatant. The supernatant fraction was decanted and filtered through a 0.2 micron filter to remove large particles and subjected to anion exchange chromatography. Bound proteins were eluted with 1.0 M NaCl, dialyzed and concentrated using Biomax™ (Millipore Corp, Bedford, MA) concentrators with a molecular mass cut-off of 50,000 Daltons. The concentrated fraction was subjected to gel filtration chromatography using Sepharose CL-4B beaded matrix. Bioassay data for material prepared in this way is shown in Table 30 and is denoted as " TcbA Cell Sup".

In yet another method to handle large amounts of material, the cell pellets were re-suspended in 10 mM sodium phosphate buffer, pH = 7.0 and thoroughly homogenized by using a Kontes Glass Company (Vineland, NJ) 40 ml tissue grinder. The cellular debris was pelleted by centrifugation at 25,000 x g and the cell supernatant was decanted, passed through a 0.2 micron filter and subjected to anion exchange chromatography using a Pharmacia 10/10 column packed with Poros HQ 50 beads. The bound proteins were eluted by performing a NaCl gradient of 0.0 to 1.0 M. Fractions containing the TcbA protein were combined and concentrated using a 50 kDa concentrator and subjected to gel filtration chromatography using Pharmacia CL-4B beaded matrix. The fractions containing TcbA oligomer, molecular mass of approximately 900 kDa, were collected and subjected to anion exchange chromatography using a Pharmacia Mono Q 10/10 column equilibrated with 20 mM Tris buffer pH = 7.3. A gradient of 0.0 to 1.0 M NaCl was used to elute recombinant TcbA protein. Recombinant TcbA eluted from the column at a salt concentration of approximately 0.3-0.4 M NaCl, the same molarity at which native TcbA oligomer is eluted from the Mono Q 10/10 column. The recombinant TcbA fraction was found to cause SCR mortality in bioassay experiments similar to those in Table 30.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Schoonover, Sue
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(ii) TITLE OF INVENTION: Insecticidal Protein Toxins From *Photorhabdus*

(iii) NUMBER OF SEQUENCES: 61

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/063,615
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(A) APPLICATION NUMBER: US 08/395,497
(B) FILING DATE: 28-FEB-1995

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(A) APPLICATION NUMBER: US 60/007,255
(B) FILING DATE: 06-NOV-1995

(vii) PRIOR APPLICATION DATA:
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(B) FILING DATE: 28-FEB-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/705,484
(B) FILING DATE: 28-AUG-1996

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Trp
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp Ala
1 5 10 15

Leu Val Ala

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Ser Pro Leu Ser Thr Ser Glu Leu Thr Ser Lys Leu Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Gly Asp Thr Ala Asn Ile Gly Asp
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Gly Gly Ala Ala Thr Leu Leu Asp Leu Leu Pro Gln Ile
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asn Leu Ala Ser Pro Leu Ile Ser
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ile Asn Leu Asp Ile Asn Glu Gln Asn Lys Ile Met Val Val Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Ala Lys Asp Val Lys Phe Gly Ser Asp Ala Arg Val Lys Met Leu
1 5 10 15

Arg Gly Val Asn
20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7515 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..7515

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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ATG CAA AAC TCA TTA TCA AGC ACT ATC GAT ACT ATT TGT CAG AAA CTG   48
Met Gln Asn Ser Leu Ser Ser Thr Ile Asp Thr Ile Cys Gln Lys Leu
  1               5                  10                  15

CAA TTA ACT TGT CCG GCG GAA ATT GCT TTG TAT CCC TTT GAT ACT TTC   96
Gln Leu Thr Cys Pro Ala Glu Ile Ala Leu Tyr Pro Phe Asp Thr Phe
             20                  25                  30

CGG GAA AAA ACT CGG GGA ATG GTT AAT TGG GGG GAA GCA AAA CGG ATT  144
Arg Glu Lys Thr Arg Gly Met Val Asn Trp Gly Glu Ala Lys Arg Ile
         35                  40                  45

TAT GAA ATT GCA CAA GCG GAA CAG GAT AGA AAC CTA CTT CAT GAA AAA  192
Tyr Glu Ile Ala Gln Ala Glu Gln Asp Arg Asn Leu Leu His Glu Lys
     50                  55                  60

CGT ATT TTT GCC TAT GCT AAT CCG CTG CTG AAA AAC GCT GTT CGG TTG  240
Arg Ile Phe Ala Tyr Ala Asn Pro Leu Leu Lys Asn Ala Val Arg Leu
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      65                  70                  75                  80

GGT ACC CGG CAA ATG TTG GGT TTT ATA CAA GGT TAT AGT GAT CTG TTT  288
Gly Thr Arg Gln Met Leu Gly Phe Ile Gln Gly Tyr Ser Asp Leu Phe
                 85                  90                  95

GGT AAT CGT GCT GAT AAC TAT GCC GCG CCG GGC TCG GTT GCA TCG ATG  336
Gly Asn Arg Ala Asp Asn Tyr Ala Ala Pro Gly Ser Val Ala Ser Met
            100                 105                 110

TTC TCA CCG GCG GCT TAT TTG ACG GAA TTG TAC CGT GAA GCC AAA AAC  384
Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asn
        115                 120                 125

TTG CAT GAC AGC AGC TCA ATT TAT TAC CTA GAT AAA CGT CGC CCG GAT  432
Leu His Asp Ser Ser Ser Ile Tyr Tyr Leu Asp Lys Arg Arg Pro Asp
    130                 135                 140

TTA GCA AGC TTA ATG CTC AGC CAG AAA AAT ATG GAT GAG GAA ATT TCA  480
Leu Ala Ser Leu Met Leu Ser Gln Lys Asn Met Asp Glu Glu Ile Ser
145                 150                 155                 160

ACG CTG GCT CTC TCT AAT GAA TTG TGC CTT GCC GGG ATC GAA ACA AAA  528
Thr Leu Ala Leu Ser Asn Glu Leu Cys Leu Ala Gly Ile Glu Thr Lys
                165                 170                 175

ACA GGA AAA TCA CAA GAT GAA GTG ATG GAT ATG TTG TCA ACT TAT CGT  576
Thr Gly Lys Ser Gln Asp Glu Val Met Asp Met Leu Ser Thr Tyr Arg
            180                 185                 190

TTA AGT GGA GAG ACA CCT TAT CAT CAC GCT TAT GAA ACT GTT CGT GAA  624
Leu Ser Gly Glu Thr Pro Tyr His His Ala Tyr Glu Thr Val Arg Glu
        195                 200                 205

ATC GTT CAT GAA CGT GAT CCA GGA TTT CGT CAT TTG TCA CAG GCA CCC  672
Ile Val His Glu Arg Asp Pro Gly Phe Arg His Leu Ser Gln Ala Pro
    210                 215                 220

ATT GTT GCT GCT AAG CTC GAT CCT GTG ACT TTG TTG GGT ATT AGC TCC  720
Ile Val Ala Ala Lys Leu Asp Pro Val Thr Leu Leu Gly Ile Ser Ser
225                 230                 235                 240

CAT ATT TCG CCA GAA CTG TAT AAC TTG CTG ATT GAG GAG ATC CCG GAA  768
His Ile Ser Pro Glu Leu Tyr Asn Leu Leu Ile Glu Glu Ile Pro Glu
                245                 250                 255

AAA GAT GAA GCC GCG CTT GAT ACG CTT TAT AAA ACA AAC TTT GGC GAT  816
Lys Asp Glu Ala Ala Leu Asp Thr Leu Tyr Lys Thr Asn Phe Gly Asp
            260                 265                 270

ATT ACT ACT GCT CAG TTA ATG TCC CCA AGT TAT CTG GCC CGG TAT TAT  864
Ile Thr Thr Ala Gln Leu Met Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr
        275                 280                 285

GGC GTC TCA CCG GAA GAT ATT GCC TAC GTG ACG ACT TCA TTA TCA CAT  912
Gly Val Ser Pro Glu Asp Ile Ala Tyr Val Thr Thr Ser Leu Ser His
    290                 295                 300

GTT GGA TAT AGC AGT GAT ATT CTG GTT ATT CCG TTG GTC GAT GGT GTG  960
Val Gly Tyr Ser Ser Asp Ile Leu Val Ile Pro Leu Val Asp Gly Val
305                 310                 315                 320

GGT AAG ATG GAA GTA GTT CGT GTT ACC CGA ACA CCA TCG GAT AAT TAT 1008
Gly Lys Met Glu Val Val Arg Val Thr Arg Thr Pro Ser Asp Asn Tyr
                325                 330                 335
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ACC AGT CAG ACG AAT TAT ATT GAG CTG TAT CCA CAG GGT GGC GAC AAT 1056
Thr Ser Gln Thr Asn Tyr Ile Glu Leu Tyr Pro Gln Gly Gly Asp Asn
            340                 345                 350

TAT TTG ATC AAA TAC AAT CTA AGC AAT AGT TTT GGT TTG GAT GAT TTT 1104
Tyr Leu Ile Lys Tyr Asn Leu Ser Asn Ser Phe Gly Leu Asp Asp Phe
        355                 360                 365

TAT CTG CAA TAT AAA GAT GGT TCC GCT GAT TGG ACT GAG ATT GCC CAT 1152
Tyr Leu Gln Tyr Lys Asp Gly Ser Ala Asp Trp Thr Glu Ile Ala His
    370                 375                 380

AAT CCC TAT CCT GAT ATG GTC ATA AAT CAA AAG TAT GAA TCA CAG GCG 1200
Asn Pro Tyr Pro Asp Met Val Ile Asn Gln Lys Tyr Glu Ser Gln Ala
385                 390                 395                 400

ACA ATC AAA CGT AGT GAC TCT GAC AAT ATA CTC AGT ATA GGG TTA CAA 1248
Thr Ile Lys Arg Ser Asp Ser Asp Asn Ile Leu Ser Ile Gly Leu Gln
                405                 410                 415

AGA TGG CAT AGC GGT AGT TAT AAT TTT GCC GCC GCC AAT TTT AAA ATT 1296
Arg Trp His Ser Gly Ser Tyr Asn Phe Ala Ala Ala Asn Phe Lys Ile
            420                 425                 430

GAC CAA TAC TCC CCG AAA GCT TTC CTG CTT AAA ATG AAT AAG GCT ATT 1344
Asp Gln Tyr Ser Pro Lys Ala Phe Leu Leu Lys Met Asn Lys Ala Ile
        435                 440                 445

CGG TTG CTC AAA GCT ACC GGC CTC TCT TTT GCT ACG TTG GAG CGT ATT 1392
Arg Leu Leu Lys Ala Thr Gly Leu Ser Phe Ala Thr Leu Glu Arg Ile
    450                 455                 460

GTT GAT AGT GTT AAT AGC ACC AAA TCC ATC ACG GTT GAG GTA TTA AAC 1440
Val Asp Ser Val Asn Ser Thr Lys Ser Ile Thr Val Glu Val Leu Asn
465                 470                 475                 480

AAG GTT TAT CGG GTA AAA TTC TAT ATT GAT CGT TAT GGC ATC AGT GAA 1488
Lys Val Tyr Arg Val Lys Phe Tyr Ile Asp Arg Tyr Gly Ile Ser Glu
                485                 490                 495

GAG ACA GCC GCT ATT TTG GCT AAT ATT AAT ATC TCT CAG CAA GCT GTT 1536
Glu Thr Ala Ala Ile Leu Ala Asn Ile Asn Ile Ser Gln Gln Ala Val
            500                 505                 510

GGC AAT CAG CTT AGC CAG TTT GAG CAA CTA TTT AAT CAC CCG CCG CTC 1584
Gly Asn Gln Leu Ser Gln Phe Glu Gln Leu Phe Asn His Pro Pro Leu
        515                 520                 525

AAT GGT ATT CGC TAT GAA ATC AGT GAG GAC AAC TCC AAA CAT CTT CCT 1632
Asn Gly Ile Arg Tyr Glu Ile Ser Glu Asp Asn Ser Lys His Leu Pro
    530                 535                 540

AAT CCT GAT CTG AAC CTT AAA CCA GAC AGT ACC GGT GAT GAT CAA CGC 1680
Asn Pro Asp Leu Asn Leu Lys Pro Asp Ser Thr Gly Asp Asp Gln Arg
545                 550                 555                 560

AAG GCG GTT TTA AAA CGC GCG TTT CAG GTT AAC GCC AGT GAG TTG TAT 1728
Lys Ala Val Leu Lys Arg Ala Phe Gln Val Asn Ala Ser Glu Leu Tyr
                565                 570                 575

CAG ATG TTA TTG ATC ACT GAT CGT AAA GAA GAC GGT GTT ATC AAA AAT 1776
Gln Met Leu Leu Ile Thr Asp Arg Lys Glu Asp Gly Val Ile Lys Asn
            580                 585                 590

AAC TTA GAG AAT TTG TCT GAT CTG TAT TTG GTT AGT TTG CTG GCC CAG 1824
Asn Leu Glu Asn Leu Ser Asp Leu Tyr Leu Val Ser Leu Leu Ala Gln
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                595                 600                 605

ATT CAT AAC CTG ACT ATT GCT GAA TTG AAC ATT TTG TTG GTG ATT TGT 1872
Ile His Asn Leu Thr Ile Ala Glu Leu Asn Ile Leu Leu Val Ile Cys
    610                 615                 620

GGC TAT GGC GAC ACC AAC ATT TAT CAG ATT ACC GAC GAT AAT TTA GCC 1920
Gly Tyr Gly Asp Thr Asn Ile Tyr Gln Ile Thr Asp Asp Asn Leu Ala
625                 630                 635                 640

AAA ATA GTG GAA ACA TTG TTG TGG ATC ACT CAA TGG TTG AAG ACC CAA 1968
Lys Ile Val Glu Thr Leu Leu Trp Ile Thr Gln Trp Leu Lys Thr Gln
                645                 650                 655

AAA TGG ACA GTT ACC GAC CTG TTT CTG ATG ACC ACG GCC ACT TAC AGC 2016
Lys Trp Thr Val Thr Asp Leu Phe Leu Met Thr Thr Ala Thr Tyr Ser
            660                 665                 670

ACC ACT TTA ACG CCA GAA ATT AGC AAT CTG ACG GCT ACG TTG TCT TCA 2064
Thr Thr Leu Thr Pro Glu Ile Ser Asn Leu Thr Ala Thr Leu Ser Ser
        675                 680                 685

ACT TTG CAT GGC AAA GAG AGT CTG ATT GGG GAA GAT CTG AAA AGA GCA 2112
Thr Leu His Gly Lys Glu Ser Leu Ile Gly Glu Asp Leu Lys Arg Ala
    690                 695                 700

ATG GCG CCT TGC TTC ACT TCG GCT TTG CAT TTG ACT TCT CAA GAA GTT 2160
Met Ala Pro Cys Phe Thr Ser Ala Leu His Leu Thr Ser Gln Glu Val
705                 710                 715                 720

GCG TAT GAC CTG CTG TTG TGG ATA GAC CAG ATT CAA CCG GCA CAA ATA 2208
Ala Tyr Asp Leu Leu Leu Trp Ile Asp Gln Ile Gln Pro Ala Gln Ile
                725                 730                 735

ACT GTT GAT GGG TTT TGG GAA GAA GTG CAA ACA ACA CCA ACC AGC TTG 2256
Thr Val Asp Gly Phe Trp Glu Glu Val Gln Thr Thr Pro Thr Ser Leu
            740                 745                 750

AAG GTG ATT ACC TTT GCT CAG GTG CTG GCA CAA TTG AGC CTG ATC TAT 2304
Lys Val Ile Thr Phe Ala Gln Val Leu Ala Gln Leu Ser Leu Ile Tyr
        755                 760                 765

CGT CGT ATT GGG TTA AGT GAA ACG GAA CTG TCA CTG ATC GTG ACT CAA 2352
Arg Arg Ile Gly Leu Ser Glu Thr Glu Leu Ser Leu Ile Val Thr Gln
    770                 775                 780

TCT TCT CTG CTA GTG GCA GGC AAA AGC ATA CTG GAT CAC GGT CTG TTA 2400
Ser Ser Leu Leu Val Ala Gly Lys Ser Ile Leu Asp His Gly Leu Leu
785                 790                 795                 800

ACC CTG ATG GCC TTG GAA GGT TTT CAT ACC TGG GTT AAT GGC TTG GGG 2448
Thr Leu Met Ala Leu Glu Gly Phe His Thr Trp Val Asn Gly Leu Gly
                805                 810                 815

CAA CAT GCC TCC TTG ATA TTG GCG GCG TTG AAA GAC GGA GCC TTG ACA 2496
Gln His Ala Ser Leu Ile Leu Ala Ala Leu Lys Asp Gly Ala Leu Thr
            820                 825                 830

GTT ACC GAT GTA GCA CAA GCT ATG AAT AAG GAG GAA TCT CTC CTA CAA 2544
Val Thr Asp Val Ala Gln Ala Met Asn Lys Glu Glu Ser Leu Leu Gln
        835                 840                 845

ATG GCA GCT AAT CAG GTG GAG AAG GAT CTA ACA AAA CTG ACC AGT TGG 2592
Met Ala Ala Asn Gln Val Glu Lys Asp Leu Thr Lys Leu Thr Ser Trp
    850                 855                 860
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ACA CAG ATT GAC GCT ATT CTG CAA TGG TTA CAG ATG TCT TCG GCC TTG 2640
Thr Gln Ile Asp Ala Ile Leu Gln Trp Leu Gln Met Ser Ser Ala Leu
865                 870                 875                 880

GCG GTT TCT CCA CTG GAT CTG GCA GGG ATG ATG GCC CTG AAA TAT GGG 2688
Ala Val Ser Pro Leu Asp Leu Ala Gly Met Met Ala Leu Lys Tyr Gly
                885                 890                 895

ATA GAT CAT AAC TAT GCT GCC TGG CAA GCT GCG GCG GCT GCG CTG ATG 2736
Ile Asp His Asn Tyr Ala Ala Trp Gln Ala Ala Ala Ala Ala Leu Met
            900                 905                 910

GCT GAT CAT GCT AAT CAG GCA CAG AAA AAA CTG GAT GAG ACG TTC AGT 2784
Ala Asp His Ala Asn Gln Ala Gln Lys Lys Leu Asp Glu Thr Phe Ser
        915                 920                 925

AAG GCA TTA TGT AAC TAT TAT ATT AAT GCT GTT GTC GAT AGT GCT GCT 2832
Lys Ala Leu Cys Asn Tyr Tyr Ile Asn Ala Val Val Asp Ser Ala Ala
    930                 935                 940

GGA GTA CGT GAT CGT AAC GGT TTA TAT ACC TAT TTG CTG ATT GAT AAT 2880
Gly Val Arg Asp Arg Asn Gly Leu Tyr Thr Tyr Leu Leu Ile Asp Asn
945                 950                 955                 960

CAG GTT TCT GCC GAT GTG ATC ACT TCA CGT ATT GCA GAA GCT ATC GCC 2928
Gln Val Ser Ala Asp Val Ile Thr Ser Arg Ile Ala Glu Ala Ile Ala
                965                 970                 975

GGT ATT CAA CTG TAC GTT AAC CGG GCT TTA AAC CGA GAT GAA GGT CAG 2976
Gly Ile Gln Leu Tyr Val Asn Arg Ala Leu Asn Arg Asp Glu Gly Gln
            980                 985                 990

CTT GCA TCG GAC GTT AGT ACC CGT CAG TTC TTC ACT GAC TGG GAA CGT 3024
Leu Ala Ser Asp Val Ser Thr Arg Gln Phe Phe Thr Asp Trp Glu Arg
        995                 1000                1005

TAC AAT AAA CGT TAC AGT ACT TGG GCT GGT GTC TCT GAA CTG GTC TAT 3072
Tyr Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr
    1010                1015                1020

TAT CCA GAA AAC TAT GTT GAT CCC ACT CAG CGC ATT GGG CAA ACC AAA 3120
Tyr Pro Glu Asn Tyr Val Asp Pro Thr Gln Arg Ile Gly Gln Thr Lys
1025                1030                1035                1040

ATG ATG GAT GCG CTG TTG CAA TCC ATC AAC CAG AGC CAG CTA AAT GCG 3168
Met Met Asp Ala Leu Leu Gln Ser Ile Asn Gln Ser Gln Leu Asn Ala
                1045                1050                1055

GAT ACG GTG GAA GAT GCT TTC AAA ACT TAT TTG ACC AGC TTT GAG CAG 3216
Asp Thr Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Ser Phe Glu Gln
            1060                1065                1070

GTA GCA AAT CTG AAA GTA ATT AGT GCT TAC CAC GAT AAT GTG AAT GTG 3264
Val Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Val Asn Val
        1075                1080                1085

GAT CAA GGA TTA ACT TAT TTT ATC GGT ATC GAC CAA GCA GCT CCG GGT 3312
Asp Gln Gly Leu Thr Tyr Phe Ile Gly Ile Asp Gln Ala Ala Pro Gly
    1090                1095                1100

ACG TAT TAC TGG CGT AGT GTT GAT CAC AGC AAA TGT GAA AAT GGC AAG 3360
Thr Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Cys Glu Asn Gly Lys
1105                1110                1115                1120

TTT GCC GCT AAT GCT TGG GGT GAG TGG AAT AAA ATT ACC TGT GCT GTC 3408
Phe Ala Ala Asn Ala Trp Gly Glu Trp Asn Lys Ile Thr Cys Ala Val
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1125 1130 1135

AAT CCT TGG AAA AAT ATC ATC CGT CCG GTT GTT TAT ATG TCC CGC TTA 3456
Asn Pro Trp Lys Asn Ile Ile Arg Pro Val Val Tyr Met Ser Arg Leu
1140 1145 1150

TAT CTG CTA TGG CTG GAG CAG CAA TCA AAG AAA AGT GAT GAT GGT AAA 3504
Tyr Leu Leu Trp Leu Glu Gln Gln Ser Lys Lys Ser Asp Asp Gly Lys
1155 1160 1165

ACC ACG ATT TAT CAA TAT AAC TTA AAA CTG GCT CAT ATT CGT TAC GAC 3552
Thr Thr Ile Tyr Gln Tyr Asn Leu Lys Leu Ala His Ile Arg Tyr Asp
1170 1175 1180

GGT AGT TGG AAT ACA CCA TTT ACT TTT GAT GTG ACA GAA AAG GTA AAA 3600
Gly Ser Trp Asn Thr Pro Phe Thr Phe Asp Val Thr Glu Lys Val Lys
1185 1190 1195 1200

AAT TAC ACG TCG AGT ACT GAT GCT GCT GAA TCT TTA GGG TTG TAT TGT 3648
Asn Tyr Thr Ser Ser Thr Asp Ala Ala Glu Ser Leu Gly Leu Tyr Cys
1205 1210 1215

ACT GGT TAT CAA GGG GAA GAC ACT CTA TTA GTT ATG TTC TAT TCG ATG 3696
Thr Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Ser Met
1220 1225 1230

CAG AGT AGT TAT AGC TCC TAT ACC GAT AAT AAT GCG CCG GTC ACT GGG 3744
Gln Ser Ser Tyr Ser Ser Tyr Thr Asp Asn Asn Ala Pro Val Thr Gly
1235 1240 1245

CTA TAT ATT TTC GCT GAT ATG TCA TCA GAC AAT ATG ACG AAT GCA CAA 3792
Leu Tyr Ile Phe Ala Asp Met Ser Ser Asp Asn Met Thr Asn Ala Gln
1250 1255 1260

GCA ACT AAC TAT TGG AAT AAC AGT TAT CCG CAA TTT GAT ACT GTG ATG 3840
Ala Thr Asn Tyr Trp Asn Asn Ser Tyr Pro Gln Phe Asp Thr Val Met
1265 1270 1275 1280

GCA GAT CCG GAT AGC GAC AAT AAA AAA GTC ATA ACC AGA AGA GTT AAT 3888
Ala Asp Pro Asp Ser Asp Asn Lys Lys Val Ile Thr Arg Arg Val Asn
1285 1290 1295

AAC CGT TAT GCG GAG GAT TAT GAA ATT CCT TCC TCT GTG ACA AGT AAC 3936
Asn Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Thr Ser Asn
1300 1305 1310

AGT AAT TAT TCT TGG GGT GAT CAC AGT TTA ACC ATG CTT TAT GGT GGT 3984
Ser Asn Tyr Ser Trp Gly Asp His Ser Leu Thr Met Leu Tyr Gly Gly
1315 1320 1325

AGT GTT CCT AAT ATT ACT TTT GAA TCG GCG GCA GAA GAT TTA AGG CTA 4032
Ser Val Pro Asn Ile Thr Phe Glu Ser Ala Ala Glu Asp Leu Arg Leu
1330 1335 1340

TCT ACC AAT ATG GCA TTG AGT ATT ATT CAT AAT GGA TAT GCG GGA ACC 4080
Ser Thr Asn Met Ala Leu Ser Ile Ile His Asn Gly Tyr Ala Gly Thr
1345 1350 1355 1360

CGC CGT ATA CAA TGT AAT CTT ATG AAA CAA TAC GCT TCA TTA GGT GAT 4128
Arg Arg Ile Gln Cys Asn Leu Met Lys Gln Tyr Ala Ser Leu Gly Asp
1365 1370 1375

AAA TTT ATA ATT TAT GAT TCA TCA TTT GAT GAT GCA AAC CGT TTT AAT 4176
Lys Phe Ile Ile Tyr Asp Ser Ser Phe Asp Asp Ala Asn Arg Phe Asn
1380 1385 1390

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CTG GTG CCA TTG TTT AAA TTC GGA AAA GAC GAG AAC TCA GAT GAT AGT 4224
Leu Val Pro Leu Phe Lys Phe Gly Lys Asp Glu Asn Ser Asp Asp Ser
        1395                1400                1405

ATT TGT ATA TAT AAT GAA AAC CCT TCC TCT GAA GAT AAG AAG TGG TAT 4272
Ile Cys Ile Tyr Asn Glu Asn Pro Ser Ser Glu Asp Lys Lys Trp Tyr
    1410                1415                1420

TTT TCT TCG AAA GAT GAC AAT AAA ACA GCG GAT TAT AAT GGT GGA ACT 4320
Phe Ser Ser Lys Asp Asp Asn Lys Thr Ala Asp Tyr Asn Gly Gly Thr
1425                1430                1435                1440

CAA TGT ATA GAT GCT GGA ACC AGT AAC AAA GAT TTT TAT TAT AAT CTC 4368
Gln Cys Ile Asp Ala Gly Thr Ser Asn Lys Asp Phe Tyr Tyr Asn Leu
                1445                1450                1455

CAG GAG ATT GAA GTA ATT AGT GTT ACT GGT GGG TAT TGG TCG AGT TAT 4416
Gln Glu Ile Glu Val Ile Ser Val Thr Gly Gly Tyr Trp Ser Ser Tyr
            1460                1465                1470

AAA ATA TCC AAC CCG ATT AAT ATC AAT ACG GGC ATT GAT AGT GCT AAA 4464
Lys Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys
        1475                1480                1485

GTA AAA GTC ACC GTA AAA GCG GGT GGT GAC GAT CAA ATC TTT ACT GCT 4512
Val Lys Val Thr Val Lys Ala Gly Gly Asp Asp Gln Ile Phe Thr Ala
    1490                1495                1500

GAT AAT AGT ACC TAT GTT CCT CAG CAA CCG GCA CCC AGT TTT GAG GAG 4560
Asp Asn Ser Thr Tyr Val Pro Gln Gln Pro Ala Pro Ser Phe Glu Glu
1505                1510                1515                1520

ATG ATT TAT CAG TTC AAT AAC CTG ACA ATA GAT TGT AAG AAT TTA AAT 4608
Met Ile Tyr Gln Phe Asn Asn Leu Thr Ile Asp Cys Lys Asn Leu Asn
                1525                1530                1535

TTC ATC GAC AAT CAG GCA CAT ATT GAG ATT GAT TTC ACC GCT ACG GCA 4656
Phe Ile Asp Asn Gln Ala His Ile Glu Ile Asp Phe Thr Ala Thr Ala
            1540                1545                1550

CAA GAT GGC CGA TTC TTG GGT GCA GAA ACT TTT ATT ATC CCG GTA ACT 4704
Gln Asp Gly Arg Phe Leu Gly Ala Glu Thr Phe Ile Ile Pro Val Thr
        1555                1560                1565

AAA AAA GTT CTC GGT ACT GAG AAC GTG ATT GCG TTA TAT AGC GAA AAT 4752
Lys Lys Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn
    1570                1575                1580

AAC GGT GTT CAA TAT ATG CAA ATT GGC GCA TAT CGT ACC CGT TTG AAT 4800
Asn Gly Val Gln Tyr Met Gln Ile Gly Ala Tyr Arg Thr Arg Leu Asn
1585                1590                1595                1600

ACG TTA TTC GCT CAA CAG TTG GTT AGC CGT GCT AAT CGT GGC ATT GAT 4848
Thr Leu Phe Ala Gln Gln Leu Val Ser Arg Ala Asn Arg Gly Ile Asp
                1605                1610                1615

GCA GTG CTC AGT ATG GAA ACT CAG AAT ATT CAG GAA CCG CAA TTA GGA 4896
Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly
            1620                1625                1630

GCG GGC ACA TAT GTG CAG CTT GTG TTG GAT AAA TAT GAT GAG TCT ATT 4944
Ala Gly Thr Tyr Val Gln Leu Val Leu Asp Lys Tyr Asp Glu Ser Ile
        1635                1640                1645

CAT GGC ACT AAT AAA AGC TTT GCT ATT GAA TAT GTT GAT ATA TTT AAA 4992
His Gly Thr Asn Lys Ser Phe Ala Ile Glu Tyr Val Asp Ile Phe Lys
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      1650                1655                1660

GAG AAC GAT AGT TTT GTG ATT TAT CAA GGA GAA CTT AGC GAA ACA AGT 5040
Glu Asn Asp Ser Phe Val Ile Tyr Gln Gly Glu Leu Ser Glu Thr Ser
1665                1670                1675                1680

CAA ACT GTT GTG AAA GTT TTC TTA TCC TAT TTT ATA GAG GCG ACT GGA 5088
Gln Thr Val Val Lys Val Phe Leu Ser Tyr Phe Ile Glu Ala Thr Gly
                1625                1690                1695

AAT AAG AAC CAC TTA TGG GTA CGT GCT AAA TAC CAA AAG GAA ACG ACT 5136
Asn Lys Asn His Leu Trp Val Arg Ala Lys Tyr Gln Lys Glu Thr Thr
            1700                1705                1710

GAT AAG ATC TTG TTC GAC CGT ACT GAT GAG AAA GAT CCG CAC GGT TGG 5184
Asp Lys Ile Leu Phe Asp Arg Thr Asp Glu Lys Asp Pro His Gly Trp
        1715                1720                1725

TTT CTC AGC GAC GAT CAC AAG ACC TTT AGT GGT CTC TCT TCC GCA CAG 5232
Phe Leu Ser Asp Asp His Lys Thr Phe Ser Gly Leu Ser Ser Ala Gln
    1730                1735                1740

GCA TTA AAG AAC GAC AGT GAA CCG ATG GAT TTC TCT GGC GCC AAT GCT 5280
Ala Leu Lys Asn Asp Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ala
1745                1750                1755                1760

CTC TAT TTC TGG GAA CTG TTC TAT TAC ACG CCG ATG ATG ATG GCT CAT 5328
Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro Met Met Met Ala His
                1765                1770                1775

CGT TTG TTG CAG GAA CAG AAT TTT GAT GCG GCG AAC CAT TGG TTC CGT 5376
Arg Leu Leu Gln Glu Gln Asn Phe Asp Ala Ala Asn His Trp Phe Arg
            1780                1785                1790

TAT GTC TGG AGT CCA TCC GGT TAT ATC GTT GAT GGT AAA ATT GCT ATC 5424
Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val Asp Gly Lys Ile Ala Ile
        1795                1800                1805

TAC CAC TGG AAC GTG CGA CCG CTG GAA GAA GAC ACC AGT TGG AAT GCA 5472
Tyr His Trp Asn Val Arg Pro Leu Glu Glu Asp Thr Ser Trp Asn Ala
    1810                1815                1820

CAA CAA CTG GAC TCC ACC GAT CCA GAT GCT GTA GCC CAA GAT GAT CCG 5520
Gln Gln Leu Asp Ser Thr Asp Pro Asp Ala Val Ala Gln Asp Asp Pro
1825                1830                1835                1840

ATG CAC TAC AAG GTG GCT ACC TTT ATG GCG ACG TTG GAT CTG CTA ATG 5568
Met His Tyr Lys Val Ala Thr Phe Met Ala Thr Leu Asp Leu Leu Met
                1845                1850                1855

GCC CGT GGT GAT GCT GCT TAC CGC CAG TTA GAG CGT GAT ACG TTG GCT 5616
Ala Arg Gly Asp Ala Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Ala
            1860                1865                1870

GAA GCT AAA ATG TGG TAT ACA CAG GCG CTT AAT CTG TTG GGT GAT GAG 5664
Glu Ala Lys Met Trp Tyr Thr Gln Ala Leu Asn Leu Leu Gly Asp Glu
        1875                1880                1885

CCA CAA GTG ATG CTG AGT ACG ACT TGG GCT AAT CCA ACA TTG GGT AAT 5712
Pro Gln Val Met Leu Ser Thr Thr Trp Ala Asn Pro Thr Leu Gly Asn
    1890                1895                1900

GCT GCT TCA AAA ACC ACA CAG CAG GTT CGT CAG CAA GTG CTT ACC CAG 5760
Ala Ala Ser Lys Thr Thr Gln Gln Val Arg Gln Gln Val Leu Thr Gln
1905                1910                1915                1920
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TTG CGT CTC AAT AGC AGG GTA AAA ACC CCG TTG CTA GGA ACA GCC AAT 5808
Leu Arg Leu Asn Ser Arg Val Lys Thr Pro Leu Leu Gly Thr Ala Asn
                1925                1930                1935

TCC CTG ACC GCT TTA TTC CTG CCG CAG GAA AAT AGC AAG CTC AAA GGC 5856
Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn Ser Lys Leu Lys Gly
            1940                1945                1950

TAC TGG CGG ACA CTG GCG CAG CGT ATG TTT AAT TTA CGT CAT AAT CTG 5904
Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn Leu Arg His Asn Leu
        1955                1960                1965

TCG ATT GAC GGC CAG CCG CTC TCC TTG CCG CTG TAT GCT AAA CCG GCT 5952
Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu Tyr Ala Lys Pro Ala
    1970                1975                1980

GAT CCA AAA GCT TTA CTG AGT GCG GCG GTT TCA GCT TCT CAA GGG GGA 6000
Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser Ala Ser Gln Gly Gly
1985                1990                1995                2000

GCC GAC TTG CCG AAG GCG CCG CTG ACT ATT CAC CGC TTC CCT CAA ATG 6048
Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His Arg Phe Pro Gln Met
                2005                2010                2015

CTA GAA GGG GCA CGG GGC TTG GTT AAC CAG CTT ATA CAG TTC GGT AGT 6096
Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu Ile Gln Phe Gly Ser
            2020                2025                2030

TCA CTA TTG GGG TAC AGT GAG CGT CAG GAT GCG GAA GCT ATG AGT CAA 6144
Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala Glu Ala Met Ser Gln
        2035                2040                2045

CTA CTG CAA ACC CAA GCC AGC GAG TTA ATA CTG ACC AGT ATT CGT ATG 6192
Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu Thr Ser Ile Arg Met
    2050                2055                2060

CAG GAT AAC CAA TTG GCA GAG CTG GAT TCG GAA AAA ACC GCC TTG CAA 6240
Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu Lys Thr Ala Leu Gln
2065                2070                2075                2080

GTC TCT TTA GCT GGA GTG CAA CAA CGG TTT GAC AGC TAT AGC CAA CTG 6288
Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp Ser Tyr Ser Gln Leu
                2085                2090                2095

TAT GAG GAG AAC ATC AAC GCA GGT GAG CAG CGA GCG CTG GCG TTA CGC 6336
Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg Ala Leu Ala Leu Arg
            2100                2105                2110

TCA GAA TCT GCT ATT GAG TCT CAG GGA GCG CAG ATT TCC CGT ATG GCA 6384
Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln Ile Ser Arg Met Ala
        2115                2120                2125

GGC GCG GGT GTT GAT ATG GCA CCA AAT ATC TTC GGC CTG GCT GAT GGC 6432
Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe Gly Leu Ala Asp Gly
    2130                2135                2140

GGC ATG CAT TAT GGT GCT ATT GCC TAT GCC ATC GCT GAC GGT ATT GAG 6480
Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile Ala Asp Gly Ile Glu
2145                2150                2155                2160

TTG AGT GCT TCT GCC AAG ATG GTT GAT GCG GAG AAA GTT GCT CAG TCG 6528
Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu Lys Val Ala Gln Ser
                2165                2170                2175

GAA ATA TAT CGC CGT CGC CGT CAA GAA TGG AAA ATT CAG CGT GAC AAC 6576
Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys Ile Gln Arg Asp Asn
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2180 2185 2190

GCA CAA GCG GAG ATT AAC CAG TTA AAC GCG CAA CTG GAA TCA CTG TCT 6624
Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln Leu Glu Ser Leu Ser
2195 2200 2205

ATT CGC CGT GAA GCC GCT GAA ATG CAA AAA GAG TAC CTG AAA ACC CAG 6672
Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu Tyr Leu Lys Thr Gln
2210 2215 2220

CAA GCT CAG GCG CAG GCA CAA CTT ACT TTC TTA AGA AGC AAA TTC AGT 6720
Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu Arg Ser Lys Phe Ser
2225 2230 2235 2240

AAT CAA GCG TTA TAT AGT TGG TTA CGA GGG CGT TTG TCA GGT ATT TAT 6768
Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser Gly Ile Tyr
2245 2250 2255

TTC CAG TTC TAT GAC TTG GCC GTA TCA CGT TGC CTG ATG GCA GAG CAA 6816
Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met Ala Glu Gln
2260 2265 2270

TCC TAT CAA TGG GAA GCT AAT GAT AAT TCC ATT AGC TTT GTC AAA CCG 6864
Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile Ser Phe Val Lys Pro
2275 2280 2285

GGT GCA TGG CAA GGA ACT TAC GCC GGC TTA TTG TGT GGA GAA GCT TTG 6912
Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu Cys Gly Glu Ala Leu
2290 2295 2300

ATA CAA AAT CTG GCA CAA ATG GAA GAG GCA TAT CTG AAA TGG GAA TCT 6960
Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr Leu Lys Trp Glu Ser
2305 2310 2315 2320

CGC GCT TTG GAA GTA GAA CGC ACG GTT TCA TTG GCA GTG GTT TAT GAT 7008
Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Val Val Tyr Asp
2325 2330 2335

TCA CTG GAA GGT AAT GAT CGT TTT AAT TTA GCG GAA CAA ATA CCT GCA 7056
Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala Glu Gln Ile Pro Ala
2340 2345 2350

TTA TTG GAT AAG GGG GAG GGA ACA GCA GGA ACT AAA GAA AAT GGG TTA 7104
Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr Lys Glu Asn Gly Leu
2355 2360 2365

TCA TTG GCT AAT GCT ATC CTG TCA GCT TCG GTC AAA TTG TCC GAC TTG 7152
Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val Lys Leu Ser Asp Leu
2370 2375 2380

AAA CTG GGA ACG GAT TAT CCA GAC AGT ATC GTT GGT AGC AAC AAG GTT 7200
Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val Gly Ser Asn Lys Val
2385 2390 2395 2400

CGT CGT ATT AAG CAA ATC AGT GTT TCG CTA CCT GCA TTG GTT GGG CCT 7248
Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro Ala Leu Val Gly Pro
2405 2410 2415

TAT CAG GAT GTT CAG GCT ATG CTC AGC TAT GGT GGC AGT ACT CAA TTG 7296
Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly Gly Ser Thr Gln Leu
2420 2425 2430

CCG AAA GGT TGT TCA GCG TTG GCT GTG TCT CAT GGT ACC AAT GAT AGT 7344
Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His Gly Thr Asn Asp Ser
2435 2440 2445

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GGT CAG TTC CAG TTG GAT TTC AAT GAC GGC AAA TAC CTG CCA TTT GAA 7392
Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys Tyr Leu Pro Phe Glu
    2450                2455                2460

GGT ATT GCT CTT GAT GAT CAG GGT ACA CTG AAT CTT CAA TTT CCG AAT 7440
Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn Leu Gln Phe Pro Asn
2465                2470                2475                2480

GCT ACC GAC AAG CAG AAA GCA ATA TTG CAA ACT ATG AGC GAT ATT ATT 7488
Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr Met Ser Asp Ile Ile
                2485                2490                2495

TTG CAT ATT CGT TAT ACC ATC CGT TAA                         7515
Leu His Ile Arg Tyr Thr Ile Arg  *
            2500                2505
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(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2505 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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Met Gln Asn Ser Leu Ser Ser Thr Ile Asp Thr Ile Cys Gln Lys Leu
 1               5                  10                  15

Gln Leu Thr Cys Pro Ala Glu Ile Ala Leu Tyr Pro Phe Asp Thr Phe
            20                  25                  30

Arg Glu Lys Thr Arg Gly Met Val Asn Trp Gly Glu Ala Lys Arg Ile
        35                  40                  45

Tyr Glu Ile Ala Gln Ala Glu Gln Asp Arg Asn Leu Leu His Glu Lys
    50                  55                  60

Arg Ile Phe Ala Tyr Ala Asn Pro Leu Leu Lys Asn Ala Val Arg Leu
65                  70                  75                  80

Gly Thr Arg Gln Met Leu Gly Phe Ile Gln Gly Tyr Ser Asp Leu Phe
                85                  90                  95

Gly Asn Arg Ala Asp Asn Tyr Ala Ala Pro Gly Ser Val Ala Ser Met
            100                 105                 110

Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asn
        115                 120                 125

Leu His Asp Ser Ser Ser Ile Tyr Tyr Leu Asp Lys Arg Arg Pro Asp
    130                 135                 140

Leu Ala Ser Leu Met Leu Ser Gln Lys Asn Met Asp Glu Glu Ile Ser
145                 150                 155                 160

Thr Leu Ala Leu Ser Asn Glu Leu Cys Leu Ala Gly Ile Glu Thr Lys
                165                 170                 175

Thr Gly Lys Ser Gln Asp Glu Val Met Asp Met Leu Ser Thr Tyr Arg
            180                 185                 190
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Leu Ser Gly Glu Thr Pro Tyr His His Ala Tyr Glu Thr Val Arg Glu
        195                 200                 205

Ile Val His Glu Arg Asp Pro Gly Phe Arg His Leu Ser Gln Ala Pro
    210                 215                 220

Ile Val Ala Ala Lys Leu Asp Pro Val Thr Leu Leu Gly Ile Ser Ser
225                 230                 235                 240

His Ile Ser Pro Glu Leu Tyr Asn Leu Leu Ile Glu Glu Ile Pro Glu
                245                 250                 255

Lys Asp Glu Ala Ala Leu Asp Thr Leu Tyr Lys Thr Asn Phe Gly Asp
            260                 265                 270

Ile Thr Thr Ala Gln Leu Met Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr
        275                 280                 285

Gly Val Ser Pro Glu Asp Ile Ala Tyr Val Thr Thr Ser Leu Ser His
    290                 295                 300

Val Gly Tyr Ser Ser Asp Ile Leu Val Ile Pro Leu Val Asp Gly Val
305                 310                 315                 320

Gly Lys Met Glu Val Val Arg Val Thr Arg Thr Pro Ser Asp Asn Tyr
                325                 330                 335

Thr Ser Gln Thr Asn Tyr Ile Glu Leu Tyr Pro Gln Gly Gly Asp Asn
            340                 345                 350

Tyr Leu Ile Lys Tyr Asn Leu Ser Asn Ser Phe Gly Leu Asp Asp Phe
        355                 360                 365

Tyr Leu Gln Tyr Lys Asp Gly Ser Ala Asp Trp Thr Glu Ile Ala His
    370                 375                 380

Asn Pro Tyr Pro Asp Met Val Ile Asn Gln Lys Tyr Glu Ser Gln Ala
385                 390                 395                 400

Thr Ile Lys Arg Ser Asp Ser Asp Asn Ile Leu Ser Ile Gly Leu Gln
                405                 410                 415

Arg Trp His Ser Gly Ser Tyr Asn Phe Ala Ala Ala Asn Phe Lys Ile
            420                 425                 430

Asp Gln Tyr Ser Pro Lys Ala Phe Leu Leu Lys Met Asn Lys Ala Ile
        435                 440                 445

Arg Leu Leu Lys Ala Thr Gly Leu Ser Phe Ala Thr Leu Glu Arg Ile
    450                 455                 460

Val Asp Ser Val Asn Ser Thr Lys Ser Ile Thr Val Glu Val Leu Asn
465                 470                 475                 480

Lys Val Tyr Arg Val Lys Phe Tyr Ile Asp Arg Tyr Gly Ile Ser Glu
                485                 490                 495

Glu Thr Ala Ala Ile Leu Ala Asn Ile Asn Ile Ser Gln Gln Ala Val
            500                 505                 510

Gly Asn Gln Leu Ser Gln Phe Glu Gln Leu Phe Asn His Pro Pro Leu
        515                 520                 525

Asn Gly Ile Arg Tyr Glu Ile Ser Glu Asp Asn Ser Lys His Leu Pro
    530                 535                 540
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Asn Pro Asp Leu Asn Leu Lys Pro Asp Ser Thr Gly Asp Asp Gln Arg
545                 550                 555                 560

Lys Ala Val Leu Lys Arg Ala Phe Gln Val Asn Ala Ser Glu Leu Tyr
                565                 570                 575

Gln Met Leu Leu Ile Thr Asp Arg Lys Glu Asp Gly Val Ile Lys Asn
            580                 585                 590

Asn Leu Glu Asn Leu Ser Asp Leu Tyr Leu Val Ser Leu Leu Ala Gln
        595                 600                 605

Ile His Asn Leu Thr Ile Ala Glu Leu Asn Ile Leu Leu Val Ile Cys
    610                 615                 620

Gly Tyr Gly Asp Thr Asn Ile Tyr Gln Ile Thr Asp Asp Asn Leu Ala
625                 630                 635                 640

Lys Ile Val Glu Thr Leu Leu Trp Ile Thr Gln Trp Leu Lys Thr Gln
                645                 650                 655

Lys Trp Thr Val Thr Asp Leu Phe Leu Met Thr Thr Ala Thr Tyr Ser
            660                 665                 670

Thr Thr Leu Thr Pro Glu Ile Ser Asn Leu Thr Ala Thr Leu Ser Ser
        675                 680                 685

Thr Leu His Gly Lys Glu Ser Leu Ile Gly Glu Asp Leu Lys Arg Ala
    690                 695                 700

Met Ala Pro Cys Phe Thr Ser Ala Leu His Leu Thr Ser Gln Glu Val
705                 710                 715                 720

Ala Tyr Asp Leu Leu Leu Trp Ile Asp Gln Ile Gln Pro Ala Gln Ile
                725                 730                 735

Thr Val Asp Gly Phe Trp Glu Glu Val Gln Thr Thr Pro Thr Ser Leu
            740                 745                 750

Lys Val Ile Thr Phe Ala Gln Val Leu Ala Gln Leu Ser Leu Ile Tyr
        755                 760                 765

Arg Arg Ile Gly Leu Ser Glu Thr Glu Leu Ser Leu Ile Val Thr Gln
    770                 775                 780

Ser Ser Leu Leu Val Ala Gly Lys Ser Ile Leu Asp His Gly Leu Leu
785                 790                 795                 800

Thr Leu Met Ala Leu Glu Gly Phe His Thr Trp Val Asn Gly Leu Gly
                805                 810                 815

Gln His Ala Ser Leu Ile Leu Ala Ala Leu Lys Asp Gly Ala Leu Thr
            820                 825                 830

Val Thr Asp Val Ala Gln Ala Met Asn Lys Glu Glu Ser Leu Leu Gln
        835                 840                 845

Met Ala Ala Asn Gln Val Glu Lys Asp Leu Thr Lys Leu Thr Ser Trp
    850                 855                 860

Thr Gln Ile Asp Ala Ile Leu Gln Trp Leu Gln Met Ser Ser Ala Leu
865                 870                 875                 880

Ala Val Ser Pro Leu Asp Leu Ala Gly Met Met Ala Leu Lys Tyr Gly
                885                 890                 895
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Ile Asp His Asn Tyr Ala Ala Trp Gln Ala Ala Ala Ala Ala Leu Met
900 905 910

Ala Asp His Ala Asn Gln Ala Gln Lys Lys Leu Asp Glu Thr Phe Ser
915 920 925

Lys Ala Leu Cys Asn Tyr Tyr Ile Asn Ala Val Val Asp Ser Ala Ala
930 935 940

Gly Val Arg Asp Arg Asn Gly Leu Tyr Thr Tyr Leu Leu Ile Asp Asn
945 950 955 960

Gln Val Ser Ala Asp Val Ile Thr Ser Arg Ile Ala Glu Ala Ile Ala
965 970 975

Gly Ile Gln Leu Tyr Val Asn Arg Ala Leu Asn Arg Asp Glu Gly Gln
980 985 990

Leu Ala Ser Asp Val Ser Thr Arg Gln Phe Phe Thr Asp Trp Glu Arg
995 1000 1005

Tyr Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr
1010 1015 1020

Tyr Pro Glu Asn Tyr Val Asp Pro Thr Gln Arg Ile Gly Gln Thr Lys
1025 1030 1035 1040

Met Met Asp Ala Leu Leu Gln Ser Ile Asn Gln Ser Gln Leu Asn Ala
1045 1050 1055

Asp Thr Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Ser Phe Glu Gln
1060 1065 1070

Val Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Val Asn Val
1075 1080 1085

Asp Gln Gly Leu Thr Tyr Phe Ile Gly Ile Asp Gln Ala Ala Pro Gly
1090 1095 1100

Thr Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Cys Glu Asn Gly Lys
1105 1110 1115 1120

Phe Ala Ala Asn Ala Trp Gly Glu Trp Asn Lys Ile Thr Cys Ala Val
1125 1130 1135

Asn Pro Trp Lys Asn Ile Ile Arg Pro Val Val Tyr Met Ser Arg Leu
1140 1145 1150

Tyr Leu Leu Trp Leu Glu Gln Gln Ser Lys Lys Ser Asp Asp Gly Lys
1155 1160 1165

Thr Thr Ile Tyr Gln Tyr Asn Leu Lys Leu Ala His Ile Arg Tyr Asp
1170 1175 1180

Gly Ser Trp Asn Thr Pro Phe Thr Phe Asp Val Thr Glu Lys Val Lys
1185 1190 1195 1200

Asn Tyr Thr Ser Ser Thr Asp Ala Ala Glu Ser Leu Gly Leu Tyr Cys
1205 1210 1215

Thr Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Ser Met
1220 1225 1230

Gln Ser Ser Tyr Ser Ser Tyr Thr Asp Asn Asn Ala Pro Val Thr Gly
1235 1240 1245

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Leu Tyr Ile Phe Ala Asp Met Ser Ser Asp Asn Met Thr Asn Ala Gln
     1250                1255                1260

Ala Thr Asn Tyr Trp Asn Asn Ser Tyr Pro Gln Phe Asp Thr Val Met
1265                1270                1275                1280

Ala Asp Pro Asp Ser Asp Asn Lys Lys Val Ile Thr Arg Arg Val Asn
                1285                1290                1295

Asn Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Thr Ser Asn
            1300                1305                1310

Ser Asn Tyr Ser Trp Gly Asp His Ser Leu Thr Met Leu Tyr Gly Gly
        1315                1320                1325

Ser Val Pro Asn Ile Thr Phe Glu Ser Ala Ala Glu Asp Leu Arg Leu
    1330                1335                1340

Ser Thr Asn Met Ala Leu Ser Ile Ile His Asn Gly Tyr Ala Gly Thr
1345                1350                1355                1360

Arg Arg Ile Gln Cys Asn Leu Met Lys Gln Tyr Ala Ser Leu Gly Asp
                1365                1370                1375

Lys Phe Ile Ile Tyr Asp Ser Ser Phe Asp Asp Ala Asn Arg Phe Asn
            1380                1385                1390

Leu Val Pro Leu Phe Lys Phe Gly Lys Asp Glu Asn Ser Asp Asp Ser
        1395                1400                1405

Ile Cys Ile Tyr Asn Glu Asn Pro Ser Ser Glu Asp Lys Lys Trp Tyr
    1410                1415                1420

Phe Ser Ser Lys Asp Asp Asn Lys Thr Ala Asp Tyr Asn Gly Gly Thr
1425                1430                1435                1440

Gln Cys Ile Asp Ala Gly Thr Ser Asn Lys Asp Phe Tyr Tyr Asn Leu
                1445                1450                1455

Gln Glu Ile Glu Val Ile Ser Val Thr Gly Gly Tyr Trp Ser Ser Tyr
            1460                1465                1470

Lys Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys
        1475                1480                1485

Val Lys Val Thr Val Lys Ala Gly Gly Asp Asp Gln Ile Phe Thr Ala
    1490                1495                1500

Asp Asn Ser Thr Tyr Val Pro Gln Gln Pro Ala Pro Ser Phe Glu Glu
1505                1510                1515                1520

Met Ile Tyr Gln Phe Asn Asn Leu Thr Ile Asp Cys Lys Asn Leu Asn
                1525                1530                1535

Phe Ile Asp Asn Gln Ala His Ile Glu Ile Asp Phe Thr Ala Thr Ala
            1540                1545                1550

Gln Asp Gly Arg Phe Leu Gly Ala Glu Thr Phe Ile Ile Pro Val Thr
        1555                1560                1565

Lys Lys Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn
    1570                1575                1580

Asn Gly Val Gln Tyr Met Gln Ile Gly Ala Tyr Arg Thr Arg Leu Asn
1585                1590                1595                1600
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Thr Leu Phe Ala Gln Gln Leu Val Ser Arg Ala Asn Arg Gly Ile Asp
1605 1610 1615

Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly
1620 1625 1630

Ala Gly Thr Tyr Val Gln Leu Val Leu Asp Lys Tyr Asp Glu Ser Ile
1635 1640 1645

His Gly Thr Asn Lys Ser Phe Ala Ile Glu Tyr Val Asp Ile Phe Lys
1650 1655 1660

Glu Asn Asp Ser Phe Val Ile Tyr Gln Gly Glu Leu Ser Glu Thr Ser
1665 1670 1675 1680

Gln Thr Val Val Lys Val Phe Leu Ser Tyr Phe Ile Glu Ala Thr Gly
1685 1690 1695

Asn Lys Asn His Leu Trp Val Arg Ala Lys Tyr Gln Lys Glu Thr Thr
1700 1705 1710

Asp Lys Ile Leu Phe Asp Arg Thr Asp Glu Lys Asp Pro His Gly Trp
1715 1720 1725

Phe Leu Ser Asp Asp His Lys Thr Phe Ser Gly Leu Ser Ser Ala Gln
1730 1735 1740

Ala Leu Lys Asn Asp Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ala
1745 1750 1755 1760

Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro Met Met Met Ala His
1765 1770 1775

Arg Leu Leu Gln Glu Gln Asn Phe Asp Ala Ala Asn His Trp Phe Arg
1780 1785 1790

Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val Asp Gly Lys Ile Ala Ile
1795 1800 1805

Tyr His Trp Asn Val Arg Pro Leu Glu Glu Asp Thr Ser Trp Asn Ala
1810 1815 1820

Gln Gln Leu Asp Ser Thr Asp Pro Asp Ala Val Ala Gln Asp Asp Pro
1825 1830 1835 1840

Met His Tyr Lys Val Ala Thr Phe Met Ala Thr Leu Asp Leu Leu Met
1845 1850 1855

Ala Arg Gly Asp Ala Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Ala
1860 1865 1870

Glu Ala Lys Met Trp Tyr Thr Gln Ala Leu Asn Leu Leu Gly Asp Glu
1875 1880 1885

Pro Gln Val Met Leu Ser Thr Thr Trp Ala Asn Pro Thr Leu Gly Asn
1890 1895 1900

Ala Ala Ser Lys Thr Thr Gln Gln Val Arg Gln Gln Val Leu Thr Gln
1905 1910 1915 1920

Leu Arg Leu Asn Ser Arg Val Lys Thr Pro Leu Leu Gly Thr Ala Asn
1925 1930 1935

Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn Ser Lys Leu Lys Gly
1940 1945 1950

Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn Leu Arg His Asn Leu
1955 1960 1965

Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu Tyr Ala Lys Pro Ala
1970 1975 1980

Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser Ala Ser Gln Gly Gly
1985 1990 1995 2000

Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His Arg Phe Pro Gln Met
2005 2010 2015

Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu Ile Gln Phe Gly Ser
2020 2025 2030

Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala Glu Ala Met Ser Gln
2035 2040 2045

Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu Thr Ser Ile Arg Met
2050 2055 2060

Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu Lys Thr Ala Leu Gln
2065 2070 2075 2080

Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp Ser Tyr Ser Gln Leu
2085 2090 2095

Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg Ala Leu Ala Leu Arg
2100 2105 2110

Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln Ile Ser Arg Met Ala
2115 2120 2125

Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe Gly Leu Ala Asp Gly
2130 2135 2140

Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile Ala Asp Gly Ile Glu
2145 2150 2155 2160

Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu Lys Val Ala Gln Ser
2165 2170 2175

Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys Ile Gln Arg Asp Asn
2180 2185 2190

Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln Leu Glu Ser Leu Ser
2195 2200 2205

Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu Tyr Leu Lys Thr Gln
2210 2215 2220

Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu Arg Ser Lys Phe Ser
2225 2230 2235 2240

Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser Gly Ile Tyr
2245 2250 2255

Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met Ala Glu Gln
2260 2265 2270

Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile Ser Phe Val Lys Pro
2275 2280 2285

Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu Cys Gly Glu Ala Leu
2290 2295 2300

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Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr Leu Lys Trp Glu Ser
2305                2310                2315                2320

Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Val Val Tyr Asp
                2325                2330                2335

Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala Glu Gln Ile Pro Ala
            2340                2345                2350

Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr Lys Glu Asn Gly Leu
        2355                2360                2365

Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val Lys Leu Ser Asp Leu
    2370                2375                2380

Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val Gly Ser Asn Lys Val
2385                2390                2395                2400

Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro Ala Leu Val Gly Pro
                2405                2410                2415

Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly Gly Ser Thr Gln Leu
            2420                2425                2430

Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His Gly Thr Asn Asp Ser
        2435                2440                2445

Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys Tyr Leu Pro Phe Glu
    2450                2455                2460

Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn Leu Gln Phe Pro Asn
2465                2470                2475                2480

Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr Met Ser Asp Ile Ile
                2485                2490                2495

Leu His Ile Arg Tyr Thr Ile Arg  *
            2500                2505
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Xaa Ala
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Gln Asn Ser Gln Thr Phe Ser Val Gly Glu Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Gln Asp Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Gln Asn Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Phe Asn Ile Asp Asp Val Ser Leu Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile Gly Ser
1 5 10 15

Leu Gln Leu Phe Ile
20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Tyr Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Ile Asp Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro
1 5 10 15

Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu
20 25

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Thr Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly

1 5 10 15

Val Gln Tyr Met Gln Ile
20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6005 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: RBS
(B) LOCATION: 1..9

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 16..3585
(D) OTHER INFORMATION: /product= "P8"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

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AAGAAGGAAT TGATT ATG TCT GAA TCT TTA TTT ACA CAA ACG TTG AAA GAA  51
                 Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu
                   1               5                  10

GCG CGC CGT GAT GCA TTG GTT GCT CAT TAT ATT GCT ACT CAG GTG CCC   99
Ala Arg Arg Asp Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro
          15                  20                  25

GCA GAT TTA AAA GAG AGT ATC CAG ACC GCG GAT GAT CTG TAC GAA TAT  147
Ala Asp Leu Lys Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr
      30                  35                  40

CTG TTG CTG GAT ACC AAA ATT AGC GAT CTG GTT ACT ACT TCA CCG CTG  195
Leu Leu Leu Asp Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu
 45                  50                  55                  60

TCC GAA GCG ATT GGC AGT CTG CAA TTG TTT ATT CAT CGT GCG ATA GAG  243
Ser Glu Ala Ile Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu
                    65                  70                  75

GGC TAT GAC GGC ACG CTG GCA GAC TCA GCA AAA CCC TAT TTT GCC GAT  291
Gly Tyr Asp Gly Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp
               80                  85                  90

GAA CAG TTT TTA TAT AAC TGG GAT AGT TTT AAC CAC CGT TAT AGC ACT  339
Glu Gln Phe Leu Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr
          95                  100                 105

TGG GCT GGC AAG GAA CGG TTG AAA TTC TAT GCC GGG GAT TAT ATT GAT  387
Trp Ala Gly Lys Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp
     110                 115                 120

CCA ACA TTG CGA TTG AAT AAG ACC GAG ATA TTT ACC GCA TTT GAA CAA  435
Pro Thr Leu Arg Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln
125                 130                 135                 140
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GGT ATT TCT CAA GGG AAA TTA AAA AGT GAA TTA GTC GAA TCT AAA TTA  483
Gly Ile Ser Gln Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu
                145                 150                 155

CGT GAT TAT CTA ATT AGT TAT GAC ACT TTA GCC ACC CTT GAT TAT ATT  531
Arg Asp Tyr Leu Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile
            160                 165                 170

ACT GCC TGC CAA GGC AAA GAT AAT AAA ACC ATC TTC TTT ATT GGC CGT  579
Thr Ala Cys Gln Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg
        175                 180                 185

ACA CAG AAT GCA CCC TAT GCA TTT TAT TGG CGA AAA TTA ACT TTA GTC  627
Thr Gln Asn Ala Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val
    190                 195                 200

ACT GAT GGC GGT AAG TTG AAA CCA GAT CAA TGG TCA GAG TGG CGA GCA  675
Thr Asp Gly Gly Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala
205                 210                 215                 220

ATT AAT GCC GGG ATT AGT GAG GCA TAT TCA GGG CAT GTC GAG CCT TTC  723
Ile Asn Ala Gly Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe
                225                 230                 235

TGG GAA AAT AAC AAG CTG CAC ATC CGT TGG TTT ACT ATC TCG AAA GAA  771
Trp Glu Asn Asn Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu
            240                 245                 250

GAT AAA ATA GAT TTT GTT TAT AAA AAC ATC TGG GTG ATG AGT AGC GAT  819
Asp Lys Ile Asp Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp
        255                 260                 265

TAT AGC TGG GCA TCA AAG AAA AAA ATC TTG GAA CTT TCT TTT ACT GAC  867
Tyr Ser Trp Ala Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp
    270                 275                 280

TAC AAT AGA GTT GGA GCA ACA GGA TCA TCA AGC CCG ACT GAA GTA GCT  915
Tyr Asn Arg Val Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala
285                 290                 295                 300

TCA CAA TAT GGT TCT GAT GCT CAG ATG AAT ATT TCT GAT GAT GGG ACT  963
Ser Gln Tyr Gly Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr
                305                 310                 315

GTA CTT ATT TTT CAG AAT GCC GGC GGA GCT ACT CCC AGT ACT GGA GTG 1011
Val Leu Ile Phe Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val
            320                 325                 330

ACG TTA TGT TAT GAC TCT GGC AAC GTG ATT AAG AAC CTA TCT AGT ACA 1059
Thr Leu Cys Tyr Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr
        335                 340                 345

GGA AGT GCA AAT TTA TCG TCA AAG GAT TAT GCC ACA ACT AAA TTA CGC 1107
Gly Ser Ala Asn Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg
    350                 355                 360

ATG TGT CAT GGA CAA AGT TAC AAT GAT AAT AAC TAC TGC AAT TTT ACA 1155
Met Cys His Gly Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr
365                 370                 375                 380

CTC TCT ATT AAT ACA ATA GAA TTC ACC TCC TAC GGC ACA TTC TCA TCA 1203
Leu Ser Ile Asn Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser
                385                 390                 395

GAT GGA AAA CAA TTT ACA CCA CCT TCT GGT TCT GCC ATT GAT TTA CAC 1251
Asp Gly Lys Gln Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His
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                400                 405                 410

CTC CCT AAT TAT GTA GAT CTC AAC GCG CTA TTA GAT ATT AGC CTC GAT 1299
Leu Pro Asn Tyr Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp
        415                 420                 425

TCA CTA CTT AAT TAT GAC GTT CAG GGG CAG TTT GGC GGA TCT AAT CCG 1347
Ser Leu Leu Asn Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro
    430                 435                 440

GTT GAT AAT TTC AGT GGT CCC TAT GGT ATT TAT CTA TGG GAA ATC TTC 1395
Val Asp Asn Phe Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe
445                 450                 455                 460

TTC CAT ATT CCG TTC CTT GTT ACG GTC CGT ATG CAA ACC GAA CAA CGT 1443
Phe His Ile Pro Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg
                465                 470                 475

TAC GAA GAC GCG GAC ACT TGG TAC AAA TAT ATT TTC CGC AGC GCC GGT 1491
Tyr Glu Asp Ala Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly
            480                 485                 490

TAT CGC GAT GCT AAT GGC CAG CTC ATT ATG GAT GGC AGT AAA CCA CGT 1539
Tyr Arg Asp Ala Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg
        495                 500                 505

TAT TGG AAT GTG ATG CCA TTG CAA CTG GAT ACC GCA TGG GAT ACC ACA 1587
Tyr Trp Asn Val Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr
    510                 515                 520

CAG CCC GCC ACC ACT GAT CCA GAT GTG ATC GCT ATG GCG GAC CCG ATG 1635
Gln Pro Ala Thr Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met
525                 530                 535                 540

CAT TAC AAG CTG GCG ATA TTC CTG CAT ACC CTT GAT CTA TTG ATT GCC 1683
His Tyr Lys Leu Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala
                545                 550                 555

CGA GGC GAC AGC GCT TAC CGT CAA CTT GAA CGC GAT ACT CTA GTC GAA 1731
Arg Gly Asp Ser Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu
            560                 565                 570

GCC AAA ATG TAC TAC ATT CAG GCA CAA CAG CTA CTG GGA CCG CGC CCT 1779
Ala Lys Met Tyr Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro
        575                 580                 585

GAT ATC CAT ACC ACC AAT ACT TGG CCA AAT CCC ACC TTG AGT AAA GAA 1827
Asp Ile His Thr Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu
    590                 595                 600

GCT GGC GCT ATT GCC ACA CCG ACA TTC CTC AGT TCA CCG GAG GTG ATG 1875
Ala Gly Ala Ile Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met
605                 610                 615                 620

ACG TTC GCT GCC TGG CTA AGC GCA GGC GAT ACC GCA AAT ATT GGC GAC 1923
Thr Phe Ala Ala Trp Leu Ser Ala Gly Asp Thr Ala Asn Ile Gly Asp
                625                 630                 635

GGT GAT TTC TTG CCA CCG TAC AAC GAT GTA CTA CTC GGT TAC TGG GAT 1971
Gly Asp Phe Leu Pro Pro Tyr Asn Asp Val Leu Leu Gly Tyr Trp Asp
            640                 645                 650

AAA CTT GAG TTA CGC CTA TAC AAC CTG CGC CAC AAT CTG AGT CTG GAT 2019
Lys Leu Glu Leu Arg Leu Tyr Asn Leu Arg His Asn Leu Ser Leu Asp
        655                 660                 665
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GGT CAA CCG CTA AAT CTG CCA CTG TAT GCC ACG CCG GTA GAC CCG AAA 2067
Gly Gln Pro Leu Asn Leu Pro Leu Tyr Ala Thr Pro Val Asp Pro Lys
    670                 675                 680

ACC CTG CAA CGC CAG CAA GCC GGA GGG GAC GGT ACA GGC AGT AGT CCG 2115
Thr Leu Gln Arg Gln Gln Ala Gly Gly Asp Gly Thr Gly Ser Ser Pro
685                 690                 695                 700

GCT GGT GGT CAA GGC AGT GTT CAG GGC TGG CGC TAT CCG TTA TTG GTA 2163
Ala Gly Gly Gln Gly Ser Val Gln Gly Trp Arg Tyr Pro Leu Leu Val
                705                 710                 715

GAA CGC GCC CGC TCT GCC GTG AGT TTG TTG ACT CAG TTC GGC AAC AGC 2211
Glu Arg Ala Arg Ser Ala Val Ser Leu Leu Thr Gln Phe Gly Asn Ser
            720                 725                 730

TTA CAA ACA ACG TTA GAA CAT CAG GAT AAT GAA AAA ATG ACG ATA CTG 2259
Leu Gln Thr Thr Leu Glu His Gln Asp Asn Glu Lys Met Thr Ile Leu
        735                 740                 745

TTG CAG ACT CAA CAG GAA GCC ATC CTG AAA CAT CAG CAC GAT ATA CAA 2307
Leu Gln Thr Gln Gln Glu Ala Ile Leu Lys His Gln His Asp Ile Gln
    750                 755                 760

CAA AAT AAT CTA AAA GGA TTA CAA CAC AGC CTG ACC GCA TTA CAG GCT 2355
Gln Asn Asn Leu Lys Gly Leu Gln His Ser Leu Thr Ala Leu Gln Ala
765                 770                 775                 780

AGC CGT GAT GGC GAC ACA TTG CGG CAA AAA CAT TAC AGC GAC CTG ATT 2403
Ser Arg Asp Gly Asp Thr Leu Arg Gln Lys His Tyr Ser Asp Leu Ile
                785                 790                 795

AAC GGT GGT CTA TCT GCG GCA GAA ATC GCC GGT CTG ACA CTA CGC AGC 2451
Asn Gly Gly Leu Ser Ala Ala Glu Ile Ala Gly Leu Thr Leu Arg Ser
            800                 805                 810

ACC GCC ATG ATT ACC AAT GGC GTT GCA ACG GGA TTG CTG ATT GCC GGC 2499
Thr Ala Met Ile Thr Asn Gly Val Ala Thr Gly Leu Leu Ile Ala Gly
        815                 820                 825

GGA ATC GCC AAC GCG GTA CCT AAC GTC TTC GGG CTG GCT AAC GGT GGA 2547
Gly Ile Ala Asn Ala Val Pro Asn Val Phe Gly Leu Ala Asn Gly Gly
    830                 835                 840

TCG GAA TGG GGA GCG CCA TTA ATT GGC TCC GGG CAA GCA ACC CAA GTT 2595
Ser Glu Trp Gly Ala Pro Leu Ile Gly Ser Gly Gln Ala Thr Gln Val
845                 850                 855                 860

GGC GCC GGC ATC CAG GAT CAG AGC GCG GGC ATT TCA GAA GTG ACA GCA 2643
Gly Ala Gly Ile Gln Asp Gln Ser Ala Gly Ile Ser Glu Val Thr Ala
                865                 870                 875

GGC TAT CAG CGT CGT CAG GAA GAA TGG GCA TTG CAA CGG GAT ATT GCT 2691
Gly Tyr Gln Arg Arg Gln Glu Glu Trp Ala Leu Gln Arg Asp Ile Ala
            880                 885                 890

GAT AAC GAA ATA ACC CAA CTG GAT GCC CAG ATA CAA AGC CTG CAA GAG 2739
Asp Asn Glu Ile Thr Gln Leu Asp Ala Gln Ile Gln Ser Leu Gln Glu
        895                 900                 905

CAA ATC ACG ATG GCA CAA AAA CAG ATC ACG CTC TCT GAA ACC GAA CAA 2787
Gln Ile Thr Met Ala Gln Lys Gln Ile Thr Leu Ser Glu Thr Glu Gln
    910                 915                 920

GCG AAT GCC CAA GCG ATT TAT GAC CTG CAA ACC ACT CGT TTT ACC GGG 2835
Ala Asn Ala Gln Ala Ile Tyr Asp Leu Gln Thr Thr Arg Phe Thr Gly
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925                 930                 935                 940

CAG GCA CTG TAT AAC TGG ATG GCC GGT CGT CTC TCC GCG CTC TAT TAC 2883
Gln Ala Leu Tyr Asn Trp Met Ala Gly Arg Leu Ser Ala Leu Tyr Tyr
                945                 950                 955

CAA ATG TAT GAT TCC ACT CTG CCA ATC TGT CTC CAG CCA AAA GCC GCA 2931
Gln Met Tyr Asp Ser Thr Leu Pro Ile Cys Leu Gln Pro Lys Ala Ala
            960                 965                 970

TTA GTA CAG GAA TTA GGC GAG AAA GAG AGC GAC AGT CTT TTC CAG GTT 2979
Leu Val Gln Glu Leu Gly Glu Lys Glu Ser Asp Ser Leu Phe Gln Val
        975                 980                 985

CCG GTG TGG AAT GAT CTG TGG CAA GGG CTG TTA GCA GGA GAA GGT TTA 3027
Pro Val Trp Asn Asp Leu Trp Gln Gly Leu Leu Ala Gly Glu Gly Leu
    990                 995                 1000

AGT TCA GAG CTA CAG AAA CTG GAT GCC ATC TGG CTT GCA CGT GGT GGT 3075
Ser Ser Glu Leu Gln Lys Leu Asp Ala Ile Trp Leu Ala Arg Gly Gly
1005                1010                1015                1020

ATT GGG CTA GAA GCC ATC CGC ACC GTG TCG CTG GAT ACC CTG TTT GGC 3123
Ile Gly Leu Glu Ala Ile Arg Thr Val Ser Leu Asp Thr Leu Phe Gly
                1025                1030                1035

ACA GGG ACG TTA AGT GAA AAT ATC AAT AAA GTG CTT AAC GGG GAA ACG 3171
Thr Gly Thr Leu Ser Glu Asn Ile Asn Lys Val Leu Asn Gly Glu Thr
            1040                1045                1050

GTA TCT CCA TCC GGT GGC GTC ACT CTG GCG CTG ACA GGG GAT ATC TTC 3219
Val Ser Pro Ser Gly Gly Val Thr Leu Ala Leu Thr Gly Asp Ile Phe
        1055                1060                1065

CAA GCA ACA CTG GAT TTG AGT CAG CTA GGT TTG GAT AAC TCT TAC AAC 3267
Gln Ala Thr Leu Asp Leu Ser Gln Leu Gly Leu Asp Asn Ser Tyr Asn
    1070                1075                1080

TTG GGT AAC GAG AAG AAA CGT CGT ATT AAA CGT ATC GCC GTC ACC CTG 3315
Leu Gly Asn Glu Lys Lys Arg Arg Ile Lys Arg Ile Ala Val Thr Leu
1085                1090                1095                1100

CCA ACA CTT CTG GGG CCA TAT CAA GAT CTT GAA GCC ACA CTG GTA ATG 3363
Pro Thr Leu Leu Gly Pro Tyr Gln Asp Leu Glu Ala Thr Leu Val Met
                1105                1110                1115

GGT GCG GAA ATC GCC GCC TTA TCA CAC GGT GTG AAT GAC GGA GGC CGG 3411
Gly Ala Glu Ile Ala Ala Leu Ser His Gly Val Asn Asp Gly Gly Arg
            1120                1125                1130

TTT GTT ACC GAC TTT AAC GAC AGC CGT TTT CTG CCT TTT GAA GGT CGA 3459
Phe Val Thr Asp Phe Asn Asp Ser Arg Phe Leu Pro Phe Glu Gly Arg
        1135                1140                1145

GAT GCA ACA ACC GGC ACA CTG GAG CTC AAT ATT TTC CAT GCG GGT AAA 3507
Asp Ala Thr Thr Gly Thr Leu Glu Leu Asn Ile Phe His Ala Gly Lys
    1150                1155                1160

GAG GGA ACG CAA CAC GAG TTG GTC GCG AAT CTG AGT GAC ATC ATT GTG 3555
Glu Gly Thr Gln His Glu Leu Val Ala Asn Leu Ser Asp Ile Ile Val
1165                1170                1175                1180

CAT CTG AAT TAC ATC ATT CGA GAC GCG TAA ATTTCTTTTC TTTGTCGATT   3605
His Leu Asn Tyr Ile Ile Arg Asp Ala  *
                1185                1190
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ACAGGTCCCT ATCAGGGGCC TGTTATTAAG GAGTACTTTA TGCAGTTTTC ACCAGAAGTA 3665

TCGATTACAA CGCTGTCACT TCCCAAAGGT GGCGGTGCTA TCAATGGCAT GGGAGAAGCA 3725

CTGAATGCTG CCGGCCCTGA TGGAATGGCC TCCCTATCTC TGCCATTACC CCTTTCGACC 3785

GGCAGAGGGA CGGCTCCTGG ATTATCGCTG ATTTACAGCA ACAGTGCAGG TAATGGGCCT 3845

TTCGGCATCG GCTGGCAATG CGGTGTTATG TCCATTAGCC GACGCACCCA ACATGGCATT 3905

CCACAATACG GTAATGACGA CACGTTCCTA TCCCCACAAG GCGAGGTCAT GAATATCGCC 3965

CTGAATGACC AAGGGCAACC TGATATCCGT CAAGACGTTA AAACGCTGCA AGGCGTTACC 4025

TTGCCAATTT CCTATACCGT GACCCGCTAT CAAGCCCGCC AGATCCTGGA TTTCAGTAAA 4085

ATCGAATACT GGCAACCTGC CTCCGGTCAA GAAGGACGCG CTTTCTGGCT GATATCGACA 4145

CCGGACGGGC ATCTACACAT CTTAGGGAAA ACCGCGCAGG CTTGTCTGGC AAATCCGCAA 4205

AATGACCAAC AAATCGCCCA GTGGTTGCTG GAAGAAACTG TGACGCCAGC CGGTGAACAT 4265

GTCAGCTATC AATATCGAGC CGAAGATGAA GCCCATTGTG ACGACAATGA AAAAACCGCT 4325

CATCCCAATG TTACCGCACA GCGCTATCTG GTACAGGTGA ACTACAGGCA ACATCAAACC 4385

ACAAGCCAGC CTGTTCGTAC TGGATAACGC ACCTCCCGCA CCGGAAGAGT GGCTGTTTCA 4445

TCTGGTCTTT GACCACGGTG AGCGCGTACC TCACTTCATA CCGTGCCAAC ATGGGATGCA 4505

GGTACAGCGC AATGGTCTGT ACGCCCGGAT ATCTTCTCTC GCTATGAATA TGGTTTTGAA 4565

GTGCGTACTC GCCGCTTATG TCAACAAGTG CTGATGTTTC ACCGCACCGC GCTCATGGCC 4625

GGAGAAGCCA GTACCAATGA CGCCCCGGAA CTGGTTGGAC GCTTAATACT GGAATATGAC 4685

AAAAACGCCA GCGTCACCAC GTTGATTACC ATCCGTCAAT TAAGCCATGA ATCGGACGGG 4745

AGGCCAGTCA CCCAGCCACC ACTAGAACTA GCCTGGCAAC GGTTTGATCT GGAGAAAATC 4805

CCGACATGGC AACGCTTTGA CGCACTAGAT AATTTTAACT CGCAGCAACG TTATCAACTG 4865

GTTGATCTGC GGGGAGAAGG GTTGCCAGGT ATGCTGTATC AAGATCGAGG CGCTTGGTGG 4925

TATAAAGCTC CGCAACGTCA GGAAGACGGA GACAGCAATG CCGTCACTTA CGACAAAATC 4985

GCCCCACTGC CTACCCTACC CAATTTGCAG GATAATGCCT CATTGATGGA TATCAACGGA 5045

GACGGCCAAC TGGATTGGGT TGTTACCGCC TCCGGTATTC GCGGATACCA TAGTCAGCAA 5105

CCCGATGGAA AGTGGACGCA CTTTACGCCA ATCAATGCCT TGCCCGTGGA ATATTTTCAT 5165

CCAAGCATCC AGTTCGCTGA CCTTACCGGG GCAGGCTTAT CTGATTTAGT GTTGATCGGG 5225

CCGAAAAGCG TGCGTCTATA TGCCAACCAG CGAAACGGCT GGCGTAAAGG AGAAGATGTC 5285

CCCCAATCCA CAGGTATCAC CCTGCCTGTC ACAGGGACCG ATGCCCGCAA ACTGGTGGCT 5345

TTCAGTGATA TGCTCGGTTC CGGTCAACAA CATCTGGTGG AAATCAAGGG TAATCGCGTC 5405

ACCTGTTGGC CGAATCTAGG GCATGGCCGT TTCGGTCAAC CACTAACTCT GTCAGGATTT 5465

AGCCAGCCCG AAAATAGCTT CAATCCCGAA CGGCTGTTTC TGGCGGATAT CGACGGCTCC 5525

GGCACCACCG ACCTTATCTA TGCGCAATCC GGCTCTTTGC TCATTTATCT CAACCAAAGT 5585

GGTAATCAGT TTGATGCCCC GTTGACATTA GCGTTGCCAG AAGGCGTACA ATTTGACAAC 5445

ACTTGCCAAC TTCAAGTCGC CGATATTCAG GGATTAGGGA TAGCCAGCTT GATTCTGACT 5505

GTGCCACATA TCGCGCCACA TCACTGGCGT TGTGACCTGT CACTGACCAA ACCCTGGTTG 5565

TTGAATGTAA TGAACAATAA CCGGGGCGCA CATCACACGC TACATTATCG TAGTTCCGCG 5825

CAATTCTGGT TGGATGAAAA ATTACAGCTC ACCAAAGCAG GCAAATCTCC GGCTTGTTAT 5885

CTGCCGTTTC CAATGCATTT GCTATGGTAT ACCGAAATTC AGGATGAAAT CAGCGGCAAC 5945

CGGCTCACCA GTGAAGTCAA CTACAGCCAC GGCGTCTGGG ATGGTAAAGA GCGGGAATTC 6005

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1190 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp
 1               5                  10                  15

Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys
            20                  25                  30

Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp
        35                  40                  45

Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile
    50                  55                  60

Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly
65                  70                  75                  80

Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu
                85                  90                  95

Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys
            100                 105                 110

Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg
        115                 120                 125

Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln
    130                 135                 140

Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu
145                 150                 155                 160

Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln
                165                 170                 175

Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala
            180                 185                 190

Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly
        195                 200                 205
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Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala Ile Asn Ala Gly
210                 215                 220

Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe Trp Glu Asn Asn
225                 230                 235                 240

Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu Asp Lys Il  Asp
                245                 250                 255

Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp Tyr Ser Trp Ala
            260                 265                 270

Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp Tyr Asn Arg Val
        275                 280                 285

Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala Ser Gln Tyr Gly
290                 295                 300

Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr Val Leu Ile Phe
305                 310                 315                 320

Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val Thr Leu Cys Tyr
                325                 330                 335

Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr Gly Ser Ala Asn
            340                 345                 350

Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg Met Cys His Gly
        355                 360                 365

Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr Leu Ser Ile Asn
370                 375                 380

Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser Asp Gly Lys Gln
385                 390                 395                 400

Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His Leu Pro Asn Tyr
                405                 410                 415

Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp Ser Leu Leu Asn
            420                 425                 430

Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro Val Asp Asn Phe
        435                 440                 445

Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe Phe His Ile Pro
450                 455                 460

Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg Tyr Glu Asp Ala
465                 470                 475                 480

Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala
                485                 490                 495

Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val
            500                 505                 510

Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr
        515                 520                 525

Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu
530                 535                 540

Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser
545                 550                 555                 560
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Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr
565 570 575

Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr
580 585 590

Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile
595 600 605

Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala
610 615 620

Trp Leu Ser Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu
625 630 635 640

Pro Pro Tyr Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu
645 650 655

Arg Leu Tyr Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu
660 665 670

Asn Leu Pro Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg
675 680 685

Gln Gln Ala Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln
690 695 700

Gly Ser Val Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg
705 710 715 720

Ser Ala Val Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr
725 730 735

Leu Glu His Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln
740 745 750

Gln Glu Ala Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu
755 760 765

Lys Gly Leu Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly
770 775 780

Asp Thr Leu Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu
785 790 795 800

Ser Ala Ala Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile
805 810 815

Thr Asn Gly Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn
820 825 830

Ala Val Pro Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly
835 840 845

Ala Pro Leu Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile
850 855 860

Gln Asp Gln Ser Ala Gly Ile Ser Glu Val Thr Ala Gly Tyr Gln Arg
865 870 875 880

Arg Gln Glu Glu Trp Ala Leu Gln Arg Asp Ile Ala Asp Asn Glu Ile
885 890 895

Thr Gln Leu Asp Ala Gln Ile Gln Ser Leu Gln Glu Gln Ile Thr Met
900 905 910

Ala Gln [redacted] Gln Ile Thr Leu Ser Glu Thr Glu [redacted] n Ala Asn Ala Gln
915 920 925

Ala Ile Tyr Asp Leu Gln Thr Thr Arg Phe Thr Gly Gln Ala Leu Tyr
930 935 940

Asn Trp Met Ala Gly Arg Leu Ser Ala Leu Tyr Tyr Gln Met Tyr Asp
945 950 955 960

Ser Thr Leu Pro Ile Cys Leu Gln Pro Lys Ala Ala Leu Val Gln Glu
965 970 975

Leu Gly Glu Lys Glu Ser Asp Ser Leu Phe Gln Val Pro Val Trp Asn
980 985 990

Asp Leu Trp Gln Gly Leu Leu Ala Gly Glu Gly Leu Ser Ser Glu Leu
995 1000 1005

Gln Lys Leu Asp Ala Ile Trp Leu Ala Arg Gly Gly Ile Gly Leu Glu
1010 1015 1020

Ala Ile Arg Thr Val Ser Leu Asp Thr Leu Phe Gly Thr Gly Thr Leu
1025 1030 1035 1040

Ser Glu Asn Ile Asn Lys Val Leu Asn Gly Glu Thr Val Ser Pro Ser
1045 1050 1055

Gly Gly Val Thr Leu Ala Leu Thr Gly Asp Ile Phe Gln Ala Thr Leu
1060 1065 1070

Asp Leu Ser Gln Leu Gly Leu Asp Asn Ser Tyr Asn Leu Gly Asn Glu
1075 1080 1085

Lys Lys Arg Arg Ile Lys Arg Ile Ala Val Thr Leu Pro Thr Leu Leu
1090 1095 1100

Gly Pro Tyr Gln Asp Leu Glu Ala Thr Leu Val Met Gly Ala Glu Ile
1105 1110 1115 1120

Ala Ala Leu Ser His Gly Val Asn Asp Gly Gly Arg Phe Val Thr Asp
1125 1130 1135

Phe Asn Asp Ser Arg Phe Leu Pro Phe Glu Gly Arg Asp Ala Thr Thr
1140 1145 1150

Gly Thr Leu Glu Leu Asn Ile Phe His Ala Gly Lys Glu Gly Thr Gln
1155 1160 1165

His Glu Leu Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr
1170 1175 1180

Ile Ile Arg Asp Ala *
1185 1190

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1881 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1881
(D) OTHER INFORMATION: /product= "P8"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
ATG TCT GAA TCT TTA TTT ACA CAA ACG TTG AAA GAA GCG CGC CGT GAT   48
Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp
  1               5                  10                  15

GCA TTG GTT GCT CAT TAT ATT GCT ACT CAG GTG CCC GCA GAT TTA AAA   96
Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys
            20                  25                  30

GAG AGT ATC CAG ACC GCG GAT GAT CTG TAC GAA TAT CTG TTG CTG GAT  144
Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp
        35                  40                  45

ACC AAA ATT AGC GAT CTG GTT ACT ACT TCA CCG CTG TCC GAA GCG ATT  192
Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile
    50                  55                  60

GGC AGT CTG CAA TTG TTT ATT CAT CGT GCG ATA GAG GGC TAT GAC GGC  240
Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly
65                  70                  75                  80

ACG CTG GCA GAC TCA GCA AAA CCC TAT TTT GCC GAT GAA CAG TTT TTA  288
Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu
                85                  90                  95

TAT AAC TGG GAT AGT TTT AAC CAC CGT TAT AGC ACT TGG GCT GGC AAG  336
Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys
            100                 105                 110

GAA CGG TTG AAA TTC TAT GCC GGG GAT TAT ATT GAT CCA ACA TTG CGA  384
Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg
        115                 120                 125

TTG AAT AAG ACC GAG ATA TTT ACC GCA TTT GAA CAA GGT ATT TCT CAA  432
Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln
    130                 135                 140

GGG AAA TTA AAA AGT GAA TTA GTC GAA TCT AAA TTA CGT GAT TAT CTA  480
Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu
145                 150                 155                 160

ATT AGT TAT GAC ACT TTA GCC ACC CTT GAT TAT ATT ACT GCC TGC CAA  528
Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln
                165                 170                 175

GGC AAA GAT AAT AAA ACC ATC TTC TTT ATT GGC CGT ACA CAG AAT GCA  576
Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala
            180                 185                 190

CCC TAT GCA TTT TAT TGG CGA AAA TTA ACT TTA GTC ACT GAT GGC GGT  624
Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly
        195                 200                 205

AAG TTG AAA CCA GAT CAA TGG TCA GAG TGG CGA GCA ATT AAT GCC GGG  672
Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala Ile Asn Ala Gly
    210                 215                 220
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ATT AGT GAG GCA TAT TCA GGG CAT GTC GAG CCT TTC TGG GAA AAT AAC  711
Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe Trp Glu Asn Asn
225                 230                 235                 240

AAG CTG CAC ATC CGT TGG TTT ACT ATC TCG AAA GAA GAT AAA ATA GAT  768
Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu Asp Lys Ile Asp
                245                 250                 255

TTT GTT TAT AAA AAC ATC TGG GTG ATG AGT AGC GAT TAT AGC TGG GCA  816
Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp Tyr Ser Trp Ala
            260                 265                 270

TCA AAG AAA AAA ATC TTG GAA CTT TCT TTT ACT GAC TAC AAT AGA GTT  864
Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp Tyr Asn Arg Val
        275                 280                 285

GGA GCA ACA GGA TCA TCA AGC CCG ACT GAA GTA GCT TCA CAA TAT GGT  912
Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala Ser Gln Tyr Gly
    290                 295                 300

TCT GAT GCT CAG ATG AAT ATT TCT GAT GAT GGG ACT GTA CTT ATT TTT  960
Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr Val Leu Ile Phe
305                 310                 315                 320

CAG AAT GCC GGC GGA GCT ACT CCC AGT ACT GGA GTG ACG TTA TGT TAT 1008
Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val Thr Leu Cys Tyr
                325                 330                 335

GAC TCT GGC AAC GTG ATT AAG AAC CTA TCT AGT ACA GGA AGT GCA AAT 1056
Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr Gly Ser Ala Asn
            340                 345                 350

TTA TCG TCA AAG GAT TAT GCC ACA ACT AAA TTA CGC ATG TGT CAT GGA 1104
Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg Met Cys His Gly
        355                 360                 365

CAA AGT TAC AAT GAT AAT AAC TAC TGC AAT TTT ACA CTC TCT ATT AAT 1152
Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr Leu Ser Ile Asn
    370                 375                 380

ACA ATA GAA TTC ACC TCC TAC GGC ACA TTC TCA TCA GAT GGA AAA CAA 1200
Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser Asp Gly Lys Gln
385                 390                 395                 400

TTT ACA CCA CCT TCT GGT TCT GCC ATT GAT TTA CAC CTC CCT AAT TAT 1248
Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His Leu Pro Asn Tyr
                405                 410                 415

GTA GAT CTC AAC GCG CTA TTA GAT ATT AGC CTC GAT TCA CTA CTT AAT 1296
Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp Ser Leu Leu Asn
            420                 425                 430

TAT GAC GTT CAG GGG CAG TTT GGC GGA TCT AAT CCG GTT GAT AAT TTC 1344
Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro Val Asp Asn Phe
        435                 440                 445

AGT GGT CCC TAT GGT ATT TAT CTA TGG GAA ATC TTC TTC CAT ATT CCG 1392
Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe Phe His Ile Pro
    450                 455                 460

TTC CTT GTT ACG GTC CGT ATG CAA ACC GAA CAA CGT TAC GAA GAC GCG 1440
Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg Tyr Glu Asp Ala
465                 470                 475                 480

GAC ACT TGG TAC AAA TAT ATT TTC CGC AGC GCC GGT TAT CGC GAT GCT 1488
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Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala
                485                 490                 495

AAT GGC CAG CTC ATT ATG GAT GGC AGT AAA CCA CGT TAT TGG AAT GTG 1536
Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val
            500                 505                 510

ATG CCA TTG CAA CTG GAT ACC GCA TGG GAT ACC ACA CAG CCC GCC ACC 1584
Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr
        515                 520                 525

ACT GAT CCA GAT GTG ATC GCT ATG GCG GAC CCG ATG CAT TAC AAG CTG 1632
Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu
    530                 535                 540

GCG ATA TTC CTG CAT ACC CTT GAT CTA TTG ATT GCC CGA GGC GAC AGC 1680
Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser
545                 550                 555                 560

GCT TAC CGT CAA CTT GAA CGC GAT ACT CTA GTC GAA GCC AAA ATG TAC 1728
Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr
                565                 570                 575

TAC ATT CAG GCA CAA CAG CTA CTG GGA CCG CGC CCT GAT ATC CAT ACC 1776
Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr
            580                 585                 590

ACC AAT ACT TGG CCA AAT CCC ACC TTG AGT AAA GAA GCT GGC GCT ATT 1824
Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile
        595                 600                 605

GCC ACA CCG ACA TTC CTC AGT TCA CCG GAG GTG ATG ACG TTC GCT GCC 1872
Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala
    610                 615                 620

TGG CTA AGC                                                     1881
Trp Leu Ser
625
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 627 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp
 1               5                  10                  15

Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys
            20                  25                  30

Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp
        35                  40                  45

Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile
    50                  55                  60

Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly
```

65 70 75 80

Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu
85 90 95

Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys
100 105 110

Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg
115 120 125

Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln
130 135 140

Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu
145 150 155 160

Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln
165 170 175

Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala
180 185 190

Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly
195 200 205

Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala Ile Asn Ala Gly
210 215 220

Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe Trp Glu Asn Asn
225 230 235 240

Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu Asp Lys Ile Asp
245 250 255

Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp Tyr Ser Trp Ala
260 265 270

Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp Tyr Asn Arg Val
275 280 285

Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala Ser Gln Tyr Gly
290 295 300

Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr Val Leu Ile Phe
305 310 315 320

Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val Thr Leu Cys Tyr
325 330 335

Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr Gly Ser Ala Asn
340 345 350

Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg Met Cys His Gly
355 360 365

Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr Leu Ser Ile Asn
370 375 380

Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser Asp Gly Lys Gln
385 390 395 400

Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His Leu Pro Asn Tyr
405 410 415

Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp Ser Leu Leu Asn

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                420                 425                 430

Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro Val Asp Asn Phe
        435                 440                 445

Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe Phe His Ile Pro
    450                 455                 460

Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg Tyr Glu Asp Ala
465                 470                 475                 480

Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala
                485                 490                 495

Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val
            500                 505                 510

Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr
        515                 520                 525

Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu
    530                 535                 540

Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser
545                 550                 555                 560

Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr
                565                 570                 575

Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr
            580                 585                 590

Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile
        595                 600                 605

Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala
    610                 615                 620

Trp Leu Ser
625
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1689 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1689
(D) OTHER INFORMATION: /product= "S8"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
GCA GGC GAT ACC GCA AAT ATT GGC GAC GGT GAT TTC TTG CCA CCG TAC   48
Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu Pro Pro Tyr
 1               5                  10                  15
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AAC GAT GTA CTA CTC GGT TAC TGG GAT AAA CTT GAG TTA CGC CTA TAC   96
Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu Arg Leu Tyr
                20                  25                  30

AAC CTG CGC CAC AAT CTG AGT CTG GAT GGT CAA CCG CTA AAT CTG CCA  144
Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu Asn Leu Pro
            35                  40                  45

CTG TAT GCC ACG CCG GTA GAC CCG AAA ACC CTG CAA CGC CAG CAA GCC  192
Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg Gln Gln Ala
        50                  55                  60

GGA GGG GAC GGT ACA GGC AGT AGT CCG GCT GGT GGT CAA GGC AGT GTT  240
Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln Gly Ser Val
    65                  70                  75                  80

CAG GGC TGG CGC TAT CCG TTA TTG GTA GAA CGC GCC CGC TCT GCC GTG  288
Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg Ser Ala Val
                85                  90                  95

AGT TTG TTG ACT CAG TTC GGC AAC AGC TTA CAA ACA ACG TTA GAA CAT  336
Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr Leu Glu His
            100                 105                 110

CAG GAT AAT GAA AAA ATG ACG ATA CTG TTG CAG ACT CAA CAG GAA GCC  384
Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln Gln Glu Ala
        115                 120                 125

ATC CTG AAA CAT CAG CAC GAT ATA CAA CAA AAT AAT CTA AAA GGA TTA  432
Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu Lys Gly Leu
    130                 135                 140

CAA CAC AGC CTG ACC GCA TTA CAG GCT AGC CGT GAT GGC GAC ACA TTG  480
Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly Asp Thr Leu
145                 150                 155                 160

CGG CAA AAA CAT TAC AGC GAC CTG ATT AAC GGT GGT CTA TCT GCG GCA  528
Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu Ser Ala Ala
                165                 170                 175

GAA ATC GCC GGT CTG ACA CTA CGC AGC ACC GCC ATG ATT ACC AAT GGC  576
Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile Thr Asn Gly
            180                 185                 190

GTT GCA ACG GGA TTG CTG ATT GCC GGC GGA ATC GCC AAC GCG GTA CCT  624
Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn Ala Val Pro
        195                 200                 205

AAC GTC TTC GGG CTG GCT AAC GGT GGA TCG GAA TGG GGA GCG CCA TTA  672
Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly Ala Pro Leu
    210                 215                 220

ATT GGC TCC GGG CAA GCA ACC CAA GTT GGC GCC GGC ATC CAG GAT CAG  720
Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile Gln Asp Gln
225                 230                 235                 240

AGC GCG GGC ATT TCA GAA GTG ACA GCA GGC TAT CAG CGT CGT CAG GAA  768
Ser Ala Gly Ile Ser Glu Val Thr Ala Gly Tyr Gln Arg Arg Gln Glu
                245                 250                 255

GAA TGG GCA TTG CAA CGG GAT ATT GCT GAT AAC GAA ATA ACC CAA CTG  816
Glu Trp Ala Leu Gln Arg Asp Ile Ala Asp Asn Glu Ile Thr Gln Leu
            260                 265                 270

GAT GCC CAG ATA CAA AGC CTG CAA GAG CAA ATC ACG ATG GCA CAA AAA  864
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Asp Ala Gln Ile Gln Ser Leu Gln Glu Gln Ile Thr Met Ala Gln Lys
          275                 280                 285

CAG ATC ACG CTC TCT GAA ACC GAA CAA GCG AAT GCC CAA GCG ATT TAT  912
Gln Ile Thr Leu Ser Glu Thr Glu Gln Ala Asn Ala Gln Ala Ile Tyr
    290                 295                 300

GAC CTG CAA ACC ACT CGT TTT ACC GGG CAG GCA CTG TAT AAC TGG ATG  960
Asp Leu Gln Thr Thr Arg Phe Thr Gly Gln Ala Leu Tyr Asn Trp Met
305                 310                 315                 320

GCC GGT CGT CTC TCC GCG CTC TAT TAC CAA ATG TAT GAT TCC ACT CTG 1008
Ala Gly Arg Leu Ser Ala Leu Tyr Tyr Gln Met Tyr Asp Ser Thr Leu
                325                 330                 335

CCA ATC TGT CTC CAG CCA AAA GCC GCA TTA GTA CAG GAA TTA GGC GAG 1056
Pro Ile Cys Leu Gln Pro Lys Ala Ala Leu Val Gln Glu Leu Gly Glu
            340                 345                 350

AAA GAG AGC GAC AGT CTT TTC CAG GTT CCG GTG TGG AAT GAT CTG TGG 1104
Lys Glu Ser Asp Ser Leu Phe Gln Val Pro Val Trp Asn Asp Leu Trp
        355                 360                 365

CAA GGG CTG TTA GCA GGA GAA GGT TTA AGT TCA GAG CTA CAG AAA CTG 1152
Gln Gly Leu Leu Ala Gly Glu Gly Leu Ser Ser Glu Leu Gln Lys Leu
    370                 375                 380

GAT GCC ATC TGG CTT GCA CGT GGT GGT ATT GGG CTA GAA GCC ATC CGC 1200
Asp Ala Ile Trp Leu Ala Arg Gly Gly Ile Gly Leu Glu Ala Ile Arg
385                 390                 395                 400

ACC GTG TCG CTG GAT ACC CTG TTT GGC ACA GGG ACG TTA AGT GAA AAT 1248
Thr Val Ser Leu Asp Thr Leu Phe Gly Thr Gly Thr Leu Ser Glu Asn
                405                 410                 415

ATC AAT AAA GTG CTT AAC GGG GAA ACG GTA TCT CCA TCC GGT GGC GTC 1296
Ile Asn Lys Val Leu Asn Gly Glu Thr Val Ser Pro Ser Gly Gly Val
            420                 425                 430

ACT CTG GCG CTG ACA GGG GAT ATC TTC CAA GCA ACA CTG GAT TTG AGT 1344
Thr Leu Ala Leu Thr Gly Asp Ile Phe Gln Ala Thr Leu Asp Leu Ser
        435                 440                 445

CAG CTA GGT TTG GAT AAC TCT TAC AAC TTG GGT AAC GAG AAG AAA CGT 1392
Gln Leu Gly Leu Asp Asn Ser Tyr Asn Leu Gly Asn Glu Lys Lys Arg
    450                 455                 460

CGT ATT AAA CGT ATC GCC GTC ACC CTG CCA ACA CTT CTG GGG CCA TAT 1440
Arg Ile Lys Arg Ile Ala Val Thr Leu Pro Thr Leu Leu Gly Pro Tyr
465                 470                 475                 480

CAA GAT CTT GAA GCC ACA CTG GTA ATG GGT GCG GAA ATC GCC GCC TTA 1488
Gln Asp Leu Glu Ala Thr Leu Val Met Gly Ala Glu Ile Ala Ala Leu
                485                 490                 495

TCA CAC GGT GTG AAT GAC GGA GGC CGG TTT GTT ACC GAC TTT AAC GAC 1536
Ser His Gly Val Asn Asp Gly Gly Arg Phe Val Thr Asp Phe Asn Asp
            500                 505                 510

AGC CGT TTT CTG CCT TTT GAA GGT CGA GAT GCA ACA ACC GGC ACA CTG 1584
Ser Arg Phe Leu Pro Phe Glu Gly Arg Asp Ala Thr Thr Gly Thr Leu
        515                 520                 525

GAG CTC AAT ATT TTC CAT GCG GGT AAA GAG GGA ACG CAA CAC GAG TTG 1632
Glu Leu Asn Ile Phe His Ala Gly Lys Glu Gly Thr Gln His Glu Leu
    530                 535                 540
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GTC GCG AAT CTG AGT GAC ATC ATT GTG CAT CTG AAT TAC ATC ATT CGA 1689
Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr Ile Ile Arg
545                 550                 555                 560

GAC GCG TAA                                                 1698
Asp Ala  *
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 563 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu Pro Pro Tyr
 1               5                  10                  15

Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu Arg Leu Tyr
            20                  25                  30

Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu Asn Leu Pro
        35                  40                  45

Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg Gln Gln Ala
    50                  55                  60

Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln Gly Ser Val
65                  70                  75                  80

Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg Ser Ala Val
                85                  90                  95

Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr Leu Glu His
            100                 105                 110

Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln Gln Glu Ala
        115                 120                 125

Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu Lys Gly Leu
    130                 135                 140

Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly Asp Thr Leu
145                 150                 155                 160

Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu Ser Ala Ala
                165                 170                 175

Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile Thr Asn Gly
            180                 185                 190

Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn Ala Val Pro
        195                 200                 205

Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly Ala Pro Leu
    210                 215                 220

Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile Gln Asp Gln
```

225 230 235 240

Ser Ala Gly Ile Ser Glu Val Thr Ala Gly Tyr Gln Arg Arg Gln Glu
245 250 255

Glu Trp Ala Leu Gln Arg Asp Ile Ala Asp Asn Glu Ile Thr Gln Leu
260 265 270

Asp Ala Gln Ile Gln Ser Leu Gln Glu Gln Ile Thr Met Ala Gln Lys
275 280 285

Gln Ile Thr Leu Ser Glu Thr Glu Gln Ala Asn Ala Gln Ala Ile Tyr
290 295 300

Asp Leu Gln Thr Thr Arg Phe Thr Gly Gln Ala Leu Tyr Asn Trp Met
305 310 315 320

Ala Gly Arg Leu Ser Ala Leu Tyr Tyr Gln Met Tyr Asp Ser Thr Leu
325 330 335

Pro Ile Cys Leu Gln Pro Lys Ala Ala Leu Val Gln Glu Leu Gly Glu
340 345 350

Lys Glu Ser Asp Ser Leu Phe Gln Val Pro Val Trp Asn Asp Leu Trp
355 360 365

Gln Gly Leu Leu Ala Gly Glu Gly Leu Ser Ser Glu Leu Gln Lys Leu
370 375 380

Asp Ala Ile Trp Leu Ala Arg Gly Gly Ile Gly Leu Glu Ala Ile Arg
385 390 395 400

Thr Val Ser Leu Asp Thr Leu Phe Gly Thr Gly Thr Leu Ser Glu Asn
405 410 415

Ile Asn Lys Val Leu Asn Gly Glu Thr Val Ser Pro Ser Gly Gly Val
420 425 430

Thr Leu Ala Leu Thr Gly Asp Ile Phe Gln Ala Thr Leu Asp Leu Ser
435 440 445

Gln Leu Gly Leu Asp Asn Ser Tyr Asn Leu Gly Asn Glu Lys Lys Arg
450 455 460

Arg Ile Lys Arg Ile Ala Val Thr Leu Pro Thr Leu Leu Gly Pro Tyr
465 470 475 480

Gln Asp Leu Glu Ala Thr Leu Val Met Gly Ala Glu Ile Ala Ala Leu
485 490 495

Ser His Gly Val Asn Asp Gly Gly Arg Phe Val Thr Asp Phe Asn Asp
500 505 510

Ser Arg Phe Leu Pro Phe Glu Gly Arg Asp Ala Thr Thr Gly Thr Leu
515 520 525

Glu Leu Asn Ile Phe His Ala Gly Lys Glu Gly Thr Gln His Glu Leu
530 535 540

Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr Ile Ile Arg
545 550 555 560

Asp Ala *

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4458 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..4458

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
ATG CAG GAT TCA CCA GAA GTA TCG ATT ACA ACG CTG TCA CTT CCC AAA   48
Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Leu Ser Leu Pro Lys
 1               5                  10                  15

GGT GGC GGT GCT ATC AAT GGC ATG GGA GAA GCA CTG AAT GCT GCC GGC   96
Gly Gly Gly Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly
            20                  25                  30

CCT GAT GGA ATG GCC TCC CTA TCT CTG CCA TTA CCC CTT TCG ACC GGC  144
Pro Asp Gly Met Ala Ser Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly
        35                  40                  45

AGA GGG ACG GCT CCT GGA TTA TCG CTG ATT TAC AGC AAC AGT GCA GGT  192
Arg Gly Thr Ala Pro Gly Leu Ser Leu Ile Tyr Ser Asn Ser Ala Gly
    50                  55                  60

AAT GGG CCT TTC GGC ATC GGC TGG CAA TGC GGT GTT ATG TCC ATT AGC  240
Asn Gly Pro Phe Gly Ile Gly Trp Gln Cys Gly Val Met Ser Ile Ser
65                  70                  75                  80

CGA CGC ACC CAA CAT GGC ATT CCA CAA TAC GGT AAT GAC GAC ACG TTC  288
Arg Arg Thr Gln His Gly Ile Pro Gln Tyr Gly Asn Asp Asp Thr Phe
                85                  90                  95

CTA TCC CCA CAA GGC GAG GTC ATG AAT ATC GCC CTG AAT GAC CAA GGG  336
Leu Ser Pro Gln Gly Glu Val Met Asn Ile Ala Leu Asn Asp Gln Gly
            100                 105                 110

CAA CCT GAT ATC CGT CAA GAC GTT AAA ACG CTG CAA GGC GTT ACC TTG  384
Gln Pro Asp Ile Arg Gln Asp Val Lys Thr Leu Gln Gly Val Thr Leu
        115                 120                 125

CCA ATT TCC TAT ACC GTG ACC CGC TAT CAA GCC CGC CAG ATC CTG GAT  432
Pro Ile Ser Tyr Thr Val Thr Arg Tyr Gln Ala Arg Gln Ile Leu Asp
    130                 135                 140

TTC AGT AAA ATC GAA TAC TGG CAA CCT GCC TCC GGT CAA GAA GGA CGC  480
Phe Ser Lys Ile Glu Tyr Trp Gln Pro Ala Ser Gly Gln Glu Gly Arg
145                 150                 155                 160

GCT TTC TGG CTG ATA TCG ACA CCG GAC GGG CAT CTA CAC ATC TTA GGG  528
Ala Phe Trp Leu Ile Ser Thr Pro Asp Gly His Leu His Ile Leu Gly
                165                 170                 175

AAA ACC GCG CAG GCT TGT CTG GCA AAT CCG CAA AAT GAC CAA CAA ATC  576
Lys Thr Ala Gln Ala Cys Leu Ala Asn Pro Gln Asn Asp Gln Gln Ile
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                    180                 185                 190

GCC CAG TGG TTG CTG GAA GAA ACT GTG ACG CCA GCC GGT GAA CAT GTC 624
Ala Gln Trp Leu Leu Glu Glu Thr Val Thr Pro Ala Gly Glu His Val
        195                 200                 205

AGC TAT CAA TAT CGA GCC GAA GAT GAA GCC CAT TGT GAC GAC AAT GAA 672
Ser Tyr Gln Tyr Arg Ala Glu Asp Glu Ala His Cys Asp Asp Asn Glu
    210                 215                 220

AAA ACC GCT CAT CCC AAT GTT ACC GCA CAG CGC TAT CTG GTA CAG GTG 720
Lys Thr Ala His Pro Asn Val Thr Ala Gln Arg Tyr Leu Val Gln Val
225                 230                 235                 240

AAC TAC GGC AAC ATC AAA CCA CAA GCC AGC CTG TTC GTA CTG GAT AAC 768
Asn Tyr Gly Asn Ile Lys Pro Gln Ala Ser Leu Phe Val Leu Asp Asn
                245                 250                 255

GCA CCT CCC GCA CCG GAA GAG TGG CTG TTT CAT CTG GTC TTT GAC CAC 816
Ala Pro Pro Ala Pro Glu Glu Trp Leu Phe His Leu Val Phe Asp His
            260                 265                 270

GGT GAG CGC GAT ACC TCA CTT CAT ACC GTG CCA ACA TGG GAT GCA GGT 864
Gly Glu Arg Asp Thr Ser Leu His Thr Val Pro Thr Trp Asp Ala Gly
        275                 280                 285

ACA GCG CAA TGG TCT GTA CGC CCG GAT ATC TTC TCT CGC TAT GAA TAT 912
Thr Ala Gln Trp Ser Val Arg Pro Asp Ile Phe Ser Arg Tyr Glu Tyr
    290                 295                 300

GGT TTT GAA GTG CGT ACT CGC CGC TTA TGT CAA CAA GTG CTG ATG TTT 960
Gly Phe Glu Val Arg Thr Arg Arg Leu Cys Gln Gln Val Leu Met Phe
305                 310                 315                 320

CAC CGC ACC GCG CTC ATG GCC GGA GAA GCC AGT ACC AAT GAC GCC CCG 1008
His Arg Thr Ala Leu Met Ala Gly Glu Ala Ser Thr Asn Asp Ala Pro
                325                 330                 335

GAA CTG GTT GGA CGC TTA ATA CTG GAA TAT GAC AAA AAC GCC AGC GTC 1056
Glu Leu Val Gly Arg Leu Ile Leu Glu Tyr Asp Lys Asn Ala Ser Val
            340                 345                 350

ACC ACG TTG ATT ACC ATC CGT CAA TTA AGC CAT GAA TCG GAC GGG AGG 1104
Thr Thr Leu Ile Thr Ile Arg Gln Leu Ser His Glu Ser Asp Gly Arg
        355                 360                 365

CCA GTC ACC CAG CCA CCA CTA GAA CTA GCC TGG CAA CGG TTT GAT CTG 1152
Pro Val Thr Gln Pro Pro Leu Glu Leu Ala Trp Gln Arg Phe Asp Leu
    370                 375                 380

GAG AAA ATC CCG ACA TGG CAA CGC TTT GAC GCA CTA GAT AAT TTT AAC 1200
Glu Lys Ile Pro Thr Trp Gln Arg Phe Asp Ala Leu Asp Asn Phe Asn
385                 390                 395                 400

TCG CAG CAA CGT TAT CAA CTG GTT GAT CTG CGG GGA GAA GGG TTG CCA 1248
Ser Gln Gln Arg Tyr Gln Leu Val Asp Leu Arg Gly Glu Gly Leu Pro
                405                 410                 415

GGT ATG CTG TAT CAA GAT CGA GGC GCT TGG TGG TAT AAA GCT CCG CAA 1296
Gly Met Leu Tyr Gln Asp Arg Gly Ala Trp Trp Tyr Lys Ala Pro Gln
            420                 425                 430

CGT CAG GAA GAC GGA GAC AGC AAT GCC GTC ACT TAC GAC AAA ATC GCC 1344
Arg Gln Glu Asp Gly Asp Ser Asn Ala Val Thr Tyr Asp Lys Ile Ala
        435                 440                 445
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TCA CTG CCT ACC CTA CCC AAT TTG CAG GAT AAT GCC TCA TTG ATG GAT 1392
Pro Leu Pro Thr Leu Pro Asn Leu Gln Asp Asn Ala Ser Leu Met Asp
    450                 455                 460

ATC AAC GGA GAC GGC CAA CTG GAT TGG GTT GTT ACC GCC TCC GGT ATT 1440
Ile Asn Gly Asp Gly Gln Leu Asp Trp Val Val Thr Ala Ser Gly Ile
465                 470                 475                 480

CGC GGA TAC CAT AGT CAG CAA CCC GAT GGA AAG TGG ACG CAC TTT ACG 1488
Arg Gly Tyr His Ser Gln Gln Pro Asp Gly Lys Trp Thr His Phe Thr
                485                 490                 495

CCA ATC AAT GCC TTG CCC GTG GAA TAT TTT CAT CCA AGC ATC CAG TTC 1536
Pro Ile Asn Ala Leu Pro Val Glu Tyr Phe His Pro Ser Ile Gln Phe
            500                 505                 510

GCT GAC CTT ACC GGG GCA GGC TTA TCT GAT TTA GTG TTG ATC GGG CCG 1584
Ala Asp Leu Thr Gly Ala Gly Leu Ser Asp Leu Val Leu Ile Gly Pro
        515                 520                 525

AAA AGC GTG CGT CTA TAT GCC AAC CAG CGA AAC GGC TGG CGT AAA GGA 1632
Lys Ser Val Arg Leu Tyr Ala Asn Gln Arg Asn Gly Trp Arg Lys Gly
    530                 535                 540

GAA GAT GTC CCC CAA TCC ACA GGT ATC ACC CTG CCT GTC ACA GGG ACC 1680
Glu Asp Val Pro Gln Ser Thr Gly Ile Thr Leu Pro Val Thr Gly Thr
545                 550                 555                 560

GAT GCC CGC AAA CTG GTG GCT TTC AGT GAT ATG CTC GGT TCC GGT CAA 1728
Asp Ala Arg Lys Leu Val Ala Phe Ser Asp Met Leu Gly Ser Gly Gln
                565                 570                 575

CAA CAT CTG GTG GAA ATC AAG GGT AAT CGC GTC ACC TGT TGG CCG AAT 1776
Gln His Leu Val Glu Ile Lys Gly Asn Arg Val Thr Cys Trp Pro Asn
            580                 585                 590

CTA GGG CAT GGC CGT TTC GGT CAA CCA CTA ACT CTG TCA GGA TTT AGC 1824
Leu Gly His Gly Arg Phe Gly Gln Pro Leu Thr Leu Ser Gly Phe Ser
        595                 600                 605

CAG CCC GAA AAT AGC TTC AAT CCC GAA CGG CTG TTT CTG GCG GAT ATC 1872
Gln Pro Glu Asn Ser Phe Asn Pro Glu Arg Leu Phe Leu Ala Asp Ile
    610                 615                 620

GAC GGC TCC GGC ACC ACC GAC CTT ATC TAT GCG CAA TCC GGC TCT TTG 1920
Asp Gly Ser Gly Thr Thr Asp Leu Ile Tyr Ala Gln Ser Gly Ser Leu
625                 630                 635                 640

CTC ATT TAT CTC AAC CAA AGT GGT AAT CAG TTT GAT GCC CCG TTG ACA 1968
Leu Ile Tyr Leu Asn Gln Ser Gly Asn Gln Phe Asp Ala Pro Leu Thr
                645                 650                 655

TTA GCG TTG CCA GAA GGC GTA CAA TTT GAC AAC ACT TGC CAA CTT CAA 2016
Leu Ala Leu Pro Glu Gly Val Gln Phe Asp Asn Thr Cys Gln Leu Gln
            660                 665                 670

GTC GCC GAT ATT CAG GGA TTA GGG ATA GCC AGC TTG ATT CTG ACT GTG 2064
Val Ala Asp Ile Gln Gly Leu Gly Ile Ala Ser Leu Ile Leu Thr Val
        675                 680                 685

CCA CAT ATC GCG CCA CAT CAC TGG CGT TGT GAC CTG TCA CTG ACC AAA 2112
Pro His Ile Ala Pro His His Trp Arg Cys Asp Leu Ser Leu Thr Lys
    690                 695                 700

CCC TGG TTG TTG AAT GTA ATG AAC AAT AAC CGG GGC GCA CAT CAC ACG 2160
Pro Trp Leu Leu Asn Val Met Asn Asn Asn Arg Gly Ala His His Thr
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705                 710                 715                 720

CTA CAT TAT CGT AGT TCC GCG CAA TTC TGG TTG GAT GAA AAA TTA CAG 2208
Leu His Tyr Arg Ser Ser Ala Gln Phe Trp Leu Asp Glu Lys Leu Gln
                725                 730                 735

CTC ACC AAA GCA GGC AAA TCT CCG GCT TGT TAT CTG CCG TTT CCA ATG 2256
Leu Thr Lys Ala Gly Lys Ser Pro Ala Cys Tyr Leu Pro Phe Pro Met
            740                 745                 750

CAT TTG CTA TGG TAT ACC GAA ATT CAG GAT GAA ATC AGC GGC AAC CGG 2304
His Leu Leu Trp Tyr Thr Glu Ile Gln Asp Glu Ile Ser Gly Asn Arg
        755                 760                 765

CTC ACC AGT GAA GTC AAC TAC AGC CAC GGC GTC TGG GAT GGT AAA GAG 2352
Leu Thr Ser Glu Val Asn Tyr Ser His Gly Val Trp Asp Gly Lys Glu
    770                 775                 780

CGG GAA TTC AGA GGA TTT GGC TGC ATC AAA CAG ACA GAT ACC ACA ACG 2400
Arg Glu Phe Arg Gly Phe Gly Cys Ile Lys Gln Thr Asp Thr Thr Thr
785                 790                 795                 800

TTT TCT CAC GGC ACC GCC CCC GAA CAG GCG GCA CCG TCG CTG AGT ATT 2448
Phe Ser His Gly Thr Ala Pro Glu Gln Ala Ala Pro Ser Leu Ser Ile
                805                 810                 815

AGC TGG TTT GCC ACC GGC ATG GAT GAA GTA GAC AGC CAA TTA GCT ACG 2496
Ser Trp Phe Ala Thr Gly Met Asp Glu Val Asp Ser Gln Leu Ala Thr
            820                 825                 830

GAA TAT TGG CAG GCA GAC ACG CAA GCT TAT AGC GGA TTT GAA ACC CGT 2544
Glu Tyr Trp Gln Ala Asp Thr Gln Ala Tyr Ser Gly Phe Glu Thr Arg
        835                 840                 845

TAT ACC GTC TGG GAT CAC ACC AAC CAG ACA GAC CAA GCA TTT ACC CCC 2592
Tyr Thr Val Trp Asp His Thr Asn Gln Thr Asp Gln Ala Phe Thr Pro
    850                 855                 860

AAT GAG ACA CAA CGT AAC TGG CTG ACG CGA GCG CTT AAA GGC CAA CTG 2640
Asn Glu Thr Gln Arg Asn Trp Leu Thr Arg Ala Leu Lys Gly Gln Leu
865                 870                 875                 880

CTA CGC ACT GAG CTC TAC GGT CTG GAC GGA ACA GAT AAG CAA ACA GTG 2688
Leu Arg Thr Glu Leu Tyr Gly Leu Asp Gly Thr Asp Lys Gln Thr Val
                885                 890                 895

CCT TAT ACC GTC AGT GAA TCG CGC TAT CAG GTA CGC TCT ATT CCC GTA 2736
Pro Tyr Thr Val Ser Glu Ser Arg Tyr Gln Val Arg Ser Ile Pro Val
            900                 905                 910

AAT AAA GAA ACT GAA TTA TCT GCC TGG GTG ACT GCT ATT GAA AAT CGC 2784
Asn Lys Glu Thr Glu Leu Ser Ala Trp Val Thr Ala Ile Glu Asn Arg
        915                 920                 925

AGC TAC CAC TAT GAA CGT ATC ATC ACT GAC CCA CAG TTC AGC CAG AGT 2832
Ser Tyr His Tyr Glu Arg Ile Ile Thr Asp Pro Gln Phe Ser Gln Ser
    930                 935                 940

ATC AAG TTG CAA CAC GAT ATC TTT GGT CAA TCA CTG CAA AGT GTC GAT 2880
Ile Lys Leu Gln His Asp Ile Phe Gly Gln Ser Leu Gln Ser Val Asp
945                 950                 955                 960

ATT GCC TGG CCG CGC CGC GAA AAA CCA GCA GTG AAT CCC TAC CCG CCT 2928
Ile Ala Trp Pro Arg Arg Glu Lys Pro Ala Val Asn Pro Tyr Pro Pro
                965                 970                 975
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ACT TTG CCG GAA ACG CTA TTT GAC AGC AGC TAT G.   AT CAA CAA CAA 2976
Thr Leu Pro Glu Thr Leu Phe Asp Ser Ser Tyr Asp Asp Gln Gln Gln
            980                 985                 990

CTA TTA CGT CTG GTG AGA CAA AAA AAT AGC TGG CAT CAC CTG ACT GAT 3024
Leu Leu Arg Leu Val Arg Gln Lys Asn Ser Trp His His Leu Thr Asp
        995                 1000                1005

GGG GAA AAC TGG CGA TTA GGT TTA CCG AAT GCA CAA CGC CGT GAT GTT 3072
Gly Glu Asn Trp Arg Leu Gly Leu Pro Asn Ala Gln Arg Arg Asp Val
    1010                1015                1020

TAT ACT TAT GAC CGG AGC AAA ATT CCA ACC GAA GGG ATT TCC CTT GAA 3120
Tyr Thr Tyr Asp Arg Ser Lys Ile Pro Thr Glu Gly Ile Ser Leu Glu
1025                1030                1035                1040

ATC TTG CTG AAA GAT GAT GGC CTG CTA GCA GAT GAA AAA GCG GCC GTT 3168
Ile Leu Leu Lys Asp Asp Gly Leu Leu Ala Asp Glu Lys Ala Ala Val
                1045                1050                1055

TAT CTG GGA CAA CAA CAG ACG TTT TAC ACC GCC GGT CAA GCG GAA GTC 3216
Tyr Leu Gly Gln Gln Gln Thr Phe Tyr Thr Ala Gly Gln Ala Glu Val
            1060                1065                1070

ACT CTA GAA AAA CCC ACG TTA CAA GCA CTG GTC GCG TTC CAA GAA ACC 3264
Thr Leu Glu Lys Pro Thr Leu Gln Ala Leu Val Ala Phe Gln Glu Thr
        1075                1080                1085

GCC ATG ATG GAC GAT ACC TCA TTA CAG GCG TAT GAA GGC GTG ATT GAA 3312
Ala Met Met Asp Asp Thr Ser Leu Gln Ala Tyr Glu Gly Val Ile Glu
    1090                1095                1100

GAG CAA GAG TTG AAT ACC GCG CTG ACA CAG GCC GGT TAT CAG CAA GTC 3360
Glu Gln Glu Leu Asn Thr Ala Leu Thr Gln Ala Gly Tyr Gln Gln Val
1105                1110                1115                1120

GCG CGG TTG TTT AAT ACC AGA TCA GAA AGC CCG GTA TGG GCG GCA CGG 3408
Ala Arg Leu Phe Asn Thr Arg Ser Glu Ser Pro Val Trp Ala Ala Arg
                1125                1130                1135

CAA GGT TAT ACC GAT TAC GGT GAC GCC GCA CAG TTC TGG CGG CCT CAG 3456
Gln Gly Tyr Thr Asp Tyr Gly Asp Ala Ala Gln Phe Trp Arg Pro Gln
            1140                1145                1150

GCT CAG CGT AAC TCG TTG CTG ACA GGG AAA ACC ACA CTG ACC TGG GAT 3504
Ala Gln Arg Asn Ser Leu Leu Thr Gly Lys Thr Thr Leu Thr Trp Asp
        1155                1160                1165

ACC CAT CAT TGT GTA ATA ATA CAG ACT CAA GAT GCC GCT GGA TTA ACG 3552
Thr His His Cys Val Ile Ile Gln Thr Gln Asp Ala Ala Gly Leu Thr
    1170                1175                1180

ACG CAA GCC CAT TAC GAT TAT CGT TTC CTT ACA CCG GTA CAA CTG ACA 3600
Thr Gln Ala His Tyr Asp Tyr Arg Phe Leu Thr Pro Val Gln Leu Thr
1185                1190                1195                1200

GAT ATT AAT GAT AAT CAA CAT ATT GTG ACT CTG GAC GCG CTA GGT CGC 3648
Asp Ile Asn Asp Asn Gln His Ile Val Thr Leu Asp Ala Leu Gly Arg
                1205                1210                1215

GTA ACC ACC AGC CGG TTC TGG GGC ACA GAG GCA GGA CAA GCC GCA GGC 3696
Val Thr Thr Ser Arg Phe Trp Gly Thr Glu Ala Gly Gln Ala Ala Gly
            1220                1225                1230

TAT TCC AAC CAG CCC TTC ACA CCA CCG GAC TCC GTA GAT AAA GCG CTG 3744
Tyr Ser Asn Gln Pro Phe Thr Pro Pro Asp Ser Val Asp Lys Ala Leu
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                1235                1240

     GCA TTA ACC GGC GCA CTC CCT GTT GCC CAA TGT TTA GTC TAT GCC GTT 3792
     Ala Leu Thr Gly Ala Leu Pro Val Ala Gln Cys Leu Val Tyr Ala Val
5        1250                1255                1260

     GAT AGC TGG ATG CCG TCG TTA TCT TTG TCT CAG CTT TCT CAG TCA CAA 3840
     Asp Ser Trp Met Pro Ser Leu Ser Leu Ser Gln Leu Ser Gln Ser Gln
     1265                1270                1275                1280
10
     GAA GAG GCA GAA GCG CTA TGG GCG CAA CTG CGT GCC GCT CAT ATG ATT 3888
     Glu Glu Ala Glu Ala Leu Trp Ala Gln Leu Arg Ala Ala His Met Ile
                    1285                1290                1295

15   ACC GAA GAT GGG AAA GTG TGT GCG TTA AGC GGG AAA CGA GGA ACA AGC 3936
     Thr Glu Asp Gly Lys Val Cys Ala Leu Ser Gly Lys Arg Gly Thr Ser
                1300                1305                1310

     CAT CAG AAC CTG ACG ATT CAA CTT ATT TCG CTA TTG GCA AGT ATT CCC 3984
20   His Gln Asn Leu Thr Ile Gln Leu Ile Ser Leu Leu Ala Ser Ile Pro
            1315                1320                1325

     CGT TTA CCG CCA CAT GTA CTG GGG ATC ACC ACT GAT CGC TAT GAT AGC 4032
     Arg Leu Pro Pro His Val Leu Gly Ile Thr Thr Asp Arg Tyr Asp Ser
25       1330                1335                1340

     GAT CCG CAA CAG CAG CAC CAA CAG ACG GTG AGC TTT AGT GAC GGT TTT 4080
     Asp Pro Gln Gln Gln His Gln Gln Thr Val Ser Phe Ser Asp Gly Phe
     1345                1350                1355                1360
30
     GGC CGG TTA CTC CAG AGT TCA GCT CGT CAT GAG TCA GGT GAT GCC TGG 4128
     Gly Arg Leu Leu Gln Ser Ser Ala Arg His Glu Ser Gly Asp Ala Trp
                    1365                1370                1375

35   CAA CGT AAA GAG GAT GGC GGG CTG GTC GTG GAT GCA AAT GGC GTT CTG 4176
     Gln Arg Lys Glu Asp Gly Gly Leu Val Val Asp Ala Asn Gly Val Leu
                1380                1385                1390

     GTC AGT GCC CCT ACA GAC ACC CGA TGG GCC GTT TCC GGT CGC ACA GAA 4224
40   Val Ser Ala Pro Thr Asp Thr Arg Trp Ala Val Ser Gly Arg Thr Glu
            1395                1400                1405

     TAT GAC GAC AAA GGC CAA CCT GTG CGT ACT TAT CAA CCC TAT TTT CTA 4272
     Tyr Asp Asp Lys Gly Gln Pro Val Arg Thr Tyr Gln Pro Tyr Phe Leu
45       1410                1415                1420

     AAT GAC TGG CGT TAC GTT AGT GAT GAC AGC GCA CGA GAT GAC CTG TTT 4320
     Asn Asp Trp Arg Tyr Val Ser Asp Asp Ser Ala Arg Asp Asp Leu Phe
     1425                1430                1435                1440
50
     GCC GAT ACC CAC CTT TAT GAT CCA TTG GGA CGG GAA TAC AAA GTC ATC 4368
     Ala Asp Thr His Leu Tyr Asp Pro Leu Gly Arg Glu Tyr Lys Val Ile
                    1445                1450                1455

55   ACT GCT AAG AAA TAT TTG CGA GAA AAG CTG TAC ACC CCG TGG TTT ATT 4416
     Thr Ala Lys Lys Tyr Leu Arg Glu Lys Leu Tyr Thr Pro Trp Phe Ile
                1460                1465                1470

     GTC AGT GAG GAT GAA AAC GAT ACA GCA TCA AGA ACC CCA TAG          4458
60   Val Ser Glu Asp Glu Asn Asp Thr Ala Ser Arg Thr Pro  *
            1475                1480                1485


     (2) INFORMATION FOR SEQ ID NO:32:
65
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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1486 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

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Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Leu Ser Leu Pro Lys
 1               5                  10                  15

Gly Gly Gly Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly
            20                  25                  30

Pro Asp Gly Met Ala Ser Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly
        35                  40                  45

Arg Gly Thr Ala Pro Gly Leu Ser Leu Ile Tyr Ser Asn Ser Ala Gly
    50                  55                  60

Asn Gly Pro Phe Gly Ile Gly Trp Gln Cys Gly Val Met Ser Ile Ser
65                  70                  75                  80

Arg Arg Thr Gln His Gly Ile Pro Gln Tyr Gly Asn Asp Asp Thr Phe
                85                  90                  95

Leu Ser Pro Gln Gly Glu Val Met Asn Ile Ala Leu Asn Asp Gln Gly
            100                 105                 110

Gln Pro Asp Ile Arg Gln Asp Val Lys Thr Leu Gln Gly Val Thr Leu
        115                 120                 125

Pro Ile Ser Tyr Thr Val Thr Arg Tyr Gln Ala Arg Gln Ile Leu Asp
    130                 135                 140

Phe Ser Lys Ile Glu Tyr Trp Gln Pro Ala Ser Gly Gln Glu Gly Arg
145                 150                 155                 160

Ala Phe Trp Leu Ile Ser Thr Pro Asp Gly His Leu His Ile Leu Gly
                165                 170                 175

Lys Thr Ala Gln Ala Cys Leu Ala Asn Pro Gln Asn Asp Gln Gln Ile
            180                 185                 190

Ala Gln Trp Leu Leu Glu Glu Thr Val Thr Pro Ala Gly Glu His Val
        195                 200                 205

Ser Tyr Gln Tyr Arg Ala Glu Asp Glu Ala His Cys Asp Asp Asn Glu
    210                 215                 220

Lys Thr Ala His Pro Asn Val Thr Ala Gln Arg Tyr Leu Val Gln Val
225                 230                 235                 240

Asn Tyr Gly Asn Ile Lys Pro Gln Ala Ser Leu Phe Val Leu Asp Asn
                245                 250                 255

Ala Pro Pro Ala Pro Glu Glu Trp Leu Phe His Leu Val Phe Asp His
            260                 265                 270

Gly Glu Arg Asp Thr Ser Leu His Thr Val Pro Thr Trp Asp Ala Gly
        275                 280                 285

Thr Ala Gln Trp Ser Val Arg Pro Asp Ile Phe Ser Arg Tyr Glu Tyr
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290 295 300

Gly Phe Glu Val Arg Thr Arg Arg Leu Cys Gln Gln Val Leu Met Phe
305 310 315 320

His Arg Thr Ala Leu Met Ala Gly Glu Ala Ser Thr Asn Asp Ala Pro
325 330 335

Glu Leu Val Gly Arg Leu Ile Leu Glu Tyr Asp Lys Asn Ala Ser Val
340 345 350

Thr Thr Leu Ile Thr Ile Arg Gln Leu Ser His Glu Ser Asp Gly Arg
355 360 365

Pro Val Thr Gln Pro Pro Leu Glu Leu Ala Trp Gln Arg Phe Asp Leu
370 375 380

Glu Lys Ile Pro Thr Trp Gln Arg Phe Asp Ala Leu Asp Asn Phe Asn
385 390 395 400

Ser Gln Gln Arg Tyr Gln Leu Val Asp Leu Arg Gly Glu Gly Leu Pro
405 410 415

Gly Met Leu Tyr Gln Asp Arg Gly Ala Trp Trp Tyr Lys Ala Pro Gln
420 425 430

Arg Gln Glu Asp Gly Asp Ser Asn Ala Val Thr Tyr Asp Lys Ile Ala
435 440 445

Pro Leu Pro Thr Leu Pro Asn Leu Gln Asp Asn Ala Ser Leu Met Asp
450 455 460

Ile Asn Gly Asp Gly Gln Leu Asp Trp Val Val Thr Ala Ser Gly Ile
465 470 475 480

Arg Gly Tyr His Ser Gln Gln Pro Asp Gly Lys Trp Thr His Phe Thr
485 490 495

Pro Ile Asn Ala Leu Pro Val Glu Tyr Phe His Pro Ser Ile Gln Phe
500 505 510

Ala Asp Leu Thr Gly Ala Gly Leu Ser Asp Leu Val Leu Ile Gly Pro
515 520 525

Lys Ser Val Arg Leu Tyr Ala Asn Gln Arg Asn Gly Trp Arg Lys Gly
530 535 540

Glu Asp Val Pro Gln Ser Thr Gly Ile Thr Leu Pro Val Thr Gly Thr
545 550 555 560

Asp Ala Arg Lys Leu Val Ala Phe Ser Asp Met Leu Gly Ser Gly Gln
565 570 575

Gln His Leu Val Glu Ile Lys Gly Asn Arg Val Thr Cys Trp Pro Asn
580 585 590

Leu Gly His Gly Arg Phe Gly Gln Pro Leu Thr Leu Ser Gly Phe Ser
595 600 605

Gln Pro Glu Asn Ser Phe Asn Pro Glu Arg Leu Phe Leu Ala Asp Ile
610 615 620

Asp Gly Ser Gly Thr Thr Asp Leu Ile Tyr Ala Gln Ser Gly Ser Leu
625 630 635 640

Leu Ile Tyr Leu Asn Gln Ser Gly Asn Gln Phe Asp Ala Pro Leu Thr

645 650 655

Leu Ala Leu Pro Glu Gly Val Gln Phe Asp Asn Thr Cys Gln Leu Gln
660 665 670

Val Ala Asp Ile Gln Gly Leu Gly Ile Ala Ser Leu Ile Leu Thr Val
675 680 685

Pro His Ile Ala Pro His His Trp Arg Cys Asp Leu Ser Leu Thr Lys
690 695 700

Pro Trp Leu Leu Asn Val Met Asn Asn Asn Arg Gly Ala His His Thr
705 710 715 720

Leu His Tyr Arg Ser Ser Ala Gln Phe Trp Leu Asp Glu Lys Leu Gln
725 730 735

Leu Thr Lys Ala Gly Lys Ser Pro Ala Cys Tyr Leu Pro Phe Pro Met
740 745 750

His Leu Leu Trp Tyr Thr Glu Ile Gln Asp Glu Ile Ser Gly Asn Arg
755 760 765

Leu Thr Ser Glu Val Asn Tyr Ser His Gly Val Trp Asp Gly Lys Glu
770 775 780

Arg Glu Phe Arg Gly Phe Gly Cys Ile Lys Gln Thr Asp Thr Thr Thr
785 790 795 800

Phe Ser His Gly Thr Ala Pro Glu Gln Ala Ala Pro Ser Leu Ser Ile
805 810 815

Ser Trp Phe Ala Thr Gly Met Asp Glu Val Asp Ser Gln Leu Ala Thr
820 825 830

Glu Tyr Trp Gln Ala Asp Thr Gln Ala Tyr Ser Gly Phe Glu Thr Arg
835 840 845

Tyr Thr Val Trp Asp His Thr Asn Gln Thr Asp Gln Ala Phe Thr Pro
850 855 860

Asn Glu Thr Gln Arg Asn Trp Leu Thr Arg Ala Leu Lys Gly Gln Leu
865 870 875 880

Leu Arg Thr Glu Leu Tyr Gly Leu Asp Gly Thr Asp Lys Gln Thr Val
885 890 895

Pro Tyr Thr Val Ser Glu Ser Arg Tyr Gln Val Arg Ser Ile Pro Val
900 905 910

Asn Lys Glu Thr Glu Leu Ser Ala Trp Val Thr Ala Ile Glu Asn Arg
915 920 925

Ser Tyr His Tyr Glu Arg Ile Ile Thr Asp Pro Gln Phe Ser Gln Ser
930 935 940

Ile Lys Leu Gln His Asp Ile Phe Gly Gln Ser Leu Gln Ser Val Asp
945 950 955 960

Ile Ala Trp Pro Arg Arg Glu Lys Pro Ala Val Asn Pro Tyr Pro Pro
965 970 975

Thr Leu Pro Glu Thr Leu Phe Asp Ser Ser Tyr Asp Asp Gln Gln Gln
980 985 990

Leu Leu Arg Leu Val Arg Gln Lys Asn Ser Trp His His Leu Thr Asp

995 1000 1005

Gly Glu Asn Trp Arg Leu Gly Leu Pro Asn Ala Gln Arg Arg Asp Val
1010 1015 1020

Tyr Thr Tyr Asp Arg Ser Lys Ile Pro Thr Glu Gly Ile Ser Leu Glu
1025 1030 1035 1040

Ile Leu Leu Lys Asp Asp Gly Leu Leu Ala Asp Glu Lys Ala Ala Val
1045 1050 1055

Tyr Leu Gly Gln Gln Gln Thr Phe Tyr Thr Ala Gly Gln Ala Glu Val
1060 1065 1070

Thr Leu Glu Lys Pro Thr Leu Gln Ala Leu Val Ala Phe Gln Glu Thr
1075 1080 1085

Ala Met Met Asp Asp Thr Ser Leu Gln Ala Tyr Glu Gly Val Ile Glu
1090 1095 1100

Glu Gln Glu Leu Asn Thr Ala Leu Thr Gln Ala Gly Tyr Gln Gln Val
1105 1110 1115 1120

Ala Arg Leu Phe Asn Thr Arg Ser Glu Ser Pro Val Trp Ala Ala Arg
1125 1130 1135

Gln Gly Tyr Thr Asp Tyr Gly Asp Ala Ala Gln Phe Trp Arg Pro Gln
1140 1145 1150

Ala Gln Arg Asn Ser Leu Leu Thr Gly Lys Thr Thr Leu Thr Trp Asp
1155 1160 1165

Thr His His Cys Val Ile Ile Gln Thr Gln Asp Ala Ala Gly Leu Thr
1170 1175 1180

Thr Gln Ala His Tyr Asp Tyr Arg Phe Leu Thr Pro Val Gln Leu Thr
1185 1190 1195 1200

Asp Ile Asn Asp Asn Gln His Ile Val Thr Leu Asp Ala Leu Gly Arg
1205 1210 1215

Val Thr Thr Ser Arg Phe Trp Gly Thr Glu Ala Gly Gln Ala Ala Gly
1220 1225 1230

Tyr Ser Asn Gln Pro Phe Thr Pro Pro Asp Ser Val Asp Lys Ala Leu
1235 1240 1245

Ala Leu Thr Gly Ala Leu Pro Val Ala Gln Cys Leu Val Tyr Ala Val
1250 1255 1260

Asp Ser Trp Met Pro Ser Leu Ser Leu Ser Gln Leu Ser Gln Ser Gln
1265 1270 1275 1280

Glu Glu Ala Glu Ala Leu Trp Ala Gln Leu Arg Ala Ala His Met Ile
1285 1290 1295

Thr Glu Asp Gly Lys Val Cys Ala Leu Ser Gly Lys Arg Gly Thr Ser
1300 1305 1310

His Gln Asn Leu Thr Ile Gln Leu Ile Ser Leu Leu Ala Ser Ile Pro
1315 1320 1325

Arg Leu Pro Pro His Val Leu Gly Ile Thr Thr Asp Arg Tyr Asp Ser
1330 1335 1340

Asp Pro Gln Gln Gln His Gln Gln Thr Val Ser Phe Ser Asp Gly Phe

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1345                1350                1355                1360

Gly Arg Leu Leu Gln Ser Ser Ala Arg His Glu Ser Gly Asp Ala Trp
                1365                1370                1375

Gln Arg Lys Glu Asp Gly Gly Leu Val Val Asp Ala Asn Gly Val Leu
            1380                1385                1390

Val Ser Ala Pro Thr Asp Thr Arg Trp Ala Val Ser Gly Arg Thr Glu
        1395                1400                1405

Tyr Asp Asp Lys Gly Gln Pro Val Arg Thr Tyr Gln Pro Tyr Phe Leu
    1410                1415                1420

Asn Asp Trp Arg Tyr Val Ser Asp Asp Ser Ala Arg Asp Asp Leu Phe
1425                1430                1435                1440

Ala Asp Thr His Leu Tyr Asp Pro Leu Gly Arg Glu Tyr Lys Val Ile
                1445                1450                1455

Thr Ala Lys Lys Tyr Leu Arg Glu Lys Leu Tyr Thr Pro Trp Phe Ile
            1460                1465                1470

Val Ser Glu Asp Glu Asn Asp Thr Ala Ser Arg Thr Pro
        1475                1480                1485
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(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

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ATG GTG ACT GTT ATG CAA AAT AAA ATA TCA TTT TTA TCA GGT ACA TCC  48
Met Val Thr Val Met Gln Asn Lys Ile Ser Phe Leu Ser Gly Thr Ser
1               5                  10                  15

GAA CAG CCC CTG CTT GAC GCC GGT TAT CAA AAC GTA TTT GAT ATC GCA  96
Glu Gln Pro Leu Leu Asp Ala Gly Tyr Gln Asn Val Phe Asp Ile Ala
            20                  25                  30

TCA ATC AGC CGG GCT ACT TTC GTT CAA TCC GTT CCC ACC CTG CCC GTT  144
Ser Ile Ser Arg Ala Thr Phe Val Gln Ser Val Pro Thr Leu Pro Val
        35                  40                  45

AAA GAG GCT CAT ACC GTC TAT CGT CAG GCG CGG CAA CGT GCG GAA AAT  192
Lys Glu Ala His Thr Val Tyr Arg Gln Ala Arg Gln Arg Ala Glu Asn
    50                  55                  60

CTG AAA TCC CTC TAC CGA GCC TGG CAA TTG CGT CAG GAG CCG GTT ATT  240
Leu Lys Ser Leu Tyr Arg Ala Trp Gln Leu Arg Gln Glu Pro Val Ile
65                  70                  75                  80

AAA GGG CTG GCT AAA CTT AAC CTA CAA TCC AAC GTT TCT GTG CTT CAA  288
Lys Gly Leu Ala Lys Leu Asn Leu Gln Ser Asn Val Ser Val Leu Gln
                85                  90                  95

GAT GCT TTG GTA GAG AAT ATT GGC GGT GAT GGG GAT TTC AGC GAT TTA  336
Asp Ala Leu Val Glu Asn Ile Gly Gly Asp Gly Asp Phe Ser Asp Leu
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                    100                 105                 110

ATG AAC CGT GCC AGT CAA TAT GCT GAC GCT GCC TCT ATT CAA TCC CTA  384
Met Asn Arg Ala Ser Gln Tyr Ala Asp Ala Ala Ser Ile Gln Ser Leu
          115                 120                 125

TTT TCA CCG GGC CGT TAT GCT TCC GCA CTC TAC AGA GTT GCT AAA GAT  432
Phe Ser Pro Gly Arg Tyr Ala Ser Ala Leu Tyr Arg Val Ala Lys Asp
      130                 135                 140

CTG CAT AAA TCA GAT TCC AGT TTG CAT ATT GAT AAT CGC CGC GCT GAT  480
Leu His Lys Ser Asp Ser Ser Leu His Ile Asp Asn Arg Arg Ala Asp
145                 150                 155                 160

CTG AAG GAT CTG ATA TTA AGC GAA ACG ACG ATG AAT AAA GAG GTC ACT  528
Leu Lys Asp Leu Ile Leu Ser Glu Thr Thr Met Asn Lys Glu Val Thr
                165                 170                 175

TCC CTT GAT ATC TTG TTG GAT GTG CTA CAA AAA GGC GGT AAA GAT ATT  576
Ser Leu Asp Ile Leu Leu Asp Val Leu Gln Lys Gly Gly Lys Asp Ile
            180                 185                 190

ACT GAG CTG TCC GGC GCA TTC TTC CCA ATG ACG TTA CCT TAT GAC GAT  624
Thr Glu Leu Ser Gly Ala Phe Phe Pro Met Thr Leu Pro Tyr Asp Asp
          195                 200                 205

CAT CTG TCG CAA ATC GAT TCC GCT TTA TCG GCA CAA GCC AGA ACG CTG  672
His Leu Ser Gln Ile Asp Ser Ala Leu Ser Ala Gln Ala Arg Thr Leu
      210                 215                 220

AAC GGT GTG TGG AAT ACT TTG ACA GAT ACC ACG GCA CAA GCG GTT TCA  720
Asn Gly Val Trp Asn Thr Leu Thr Asp Thr Thr Ala Gln Ala Val Ser
225                 230                 235                 240

GAA CAA ACC AGT AAT ACG AAT ACA CGC AAA CTG TTC GCT GCC CAA GAT  768
Glu Gln Thr Ser Asn Thr Asn Thr Arg Lys Leu Phe Ala Ala Gln Asp
                245                 250                 255

GGT AAT CAA GAT ACA TTT TTT TCC GGA AAC ACT TTT TAT TTC AAA GCG  816
Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr Phe Tyr Phe Lys Ala
            260                 265                 270

GTG GGA TTC AGC GGG CAA CCT ATG GTT TAC CTG TCA CAG TAC ACC AGC  864
Val Gly Phe Ser Gly Gln Pro Met Val Tyr Leu Ser Gln Tyr Thr Ser
          275                 280                 285

GGG AAC GGC ATT GTC GGC GCA CAA TTG ATT GCA GGT AAT CCA GAC CAA  912
Gly Asn Gly Ile Val Gly Ala Gln Leu Ile Ala Gly Asn Pro Asp Gln
      290                 295                 300

GCC GCC GCC GCA ATA GTC GCA CCG TTG AAA CTC ACT TGG TCA ATG GCA  960
Ala Ala Ala Ala Ile Val Ala Pro Leu Lys Leu Thr Trp Ser Met Ala
305                 310                 315                 320

AAA CAG TGT TAC TAC CTC GTC GCT CCC GAT GGT ACA ACG ATG GGA GAC 1008
Lys Gln Cys Tyr Tyr Leu Val Ala Pro Asp Gly Thr Thr Met Gly Asp
                325                 330                 335

GGT AAT GTT CTG ACC GGC TGT TTC TTA AGA GGC AAC AGC CCA ACT AAC 1056
Gly Asn Val Leu Thr Gly Cys Phe Leu Arg Gly Asn Ser Pro Thr Asn
            340                 345                 350

CCG GAT AAA GAC GGT ATT TTT GCT CAG GTA GCC AAC AAA TCA GGC AGT 1104
Pro Asp Lys Asp Gly Ile Phe Ala Gln Val Ala Asn Lys Ser Gly Ser
          355                 360                 365
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ACT CAG CCT TTG CCA AGC TTC CAT CTG CCG GTC ACA CTG GAA CAC AGC  1152
Thr Gln Pro Leu Pro Ser Phe His Leu Pro Val Thr Leu Glu His Ser
    370                 375                 380

GAG AAT AAA GAT CAG TAC TAT CTG AAA ACA GAG CAG GGT TAT ATC ACG  1200
Glu Asn Lys Asp Gln Tyr Tyr Leu Lys Thr Glu Gln Gly Tyr Ile Thr
385                 390                 395                 400

GTA GAT AGT TCC GGA CAG TCA AAT TGG AAA AAC GCG CTG GTT ATC AAT  1248
Val Asp Ser Ser Gly Gln Ser Asn Trp Lys Asn Ala Leu Val Ile Asn
                405                 410                 415

GGG ACA AAA GAC AAG GGG CTG TTA TTA ACC TTT TGC AGC GAT AGC TCA  1296
Gly Thr Lys Asp Lys Gly Leu Leu Leu Thr Phe Cys Ser Asp Ser Ser
            420                 425                 430

GGC ACT CCG ACA AAC CCT GAT GAT GTG ATT CCT CCC GCT ATC AAT GAT  1344
Gly Thr Pro Thr Asn Pro Asp Asp Val Ile Pro Pro Ala Ile Asn Asp
        435                 440                 445

ATT CCA TCG CCG CCA GCC CGC GAA ACA CTG TCA CTG ACG CCG GTC AGT  1392
Ile Pro Ser Pro Pro Ala Arg Glu Thr Leu Ser Leu Thr Pro Val Ser
    450                 455                 460

TAT CAA TTG ATG ACC AAT CCG GCA CCG ACA GAA GAT GAT ATT ACC AAC  1440
Tyr Gln Leu Met Thr Asn Pro Ala Pro Thr Glu Asp Asp Ile Thr Asn
465                 470                 475                 480

CAT TAT GGT TTT AAC GGC GCT AGC TTA CGG GCT TCT CCA TTG TCA ACC  1488
His Tyr Gly Phe Asn Gly Ala Ser Leu Arg Ala Ser Pro Leu Ser Thr
                485                 490                 495

AGC GAG TTG ACC AGC AAA CTG AAT TCT ATC GAT ACT TTC TGT GAG AAG  1536
Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr Phe Cys Glu Lys
            500                 505                 510

ACC CGG TTA AGC TTC AAT CAG TTA ATG GAT TTG ACC GCT CAG CAA TCT  1584
Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr Ala Gln Gln Ser
        515                 520                 525

TAC AGT CAA AGC AGC ATT GAT GCG AAA GCA GCC AGC CGC TAT GTT CGT  1632
Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser Arg Tyr Val Arg
    530                 535                 540

TTT GGG GAA ACC ACC CCA ACC CGC GTC AAT GTC TAC GGT GCC GCT TAT  1680
Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr Gly Ala Ala Tyr
545                 550                 555                 560

CTG AAC AGC ACA CTG GCA GAC GCG GCT GAT GGT CAA TAT CTG TGG ATT  1728
Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln Tyr Leu Trp Ile
                565                 570                 575

CAG ACT GAT GGC AAG AGC CTA AAT TTC ACT GAC GAT ACG GTA GTC GCC  1776
Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp Thr Val Val Ala
            580                 585                 590

TTA GCC GGT CGC GCT GAA AAG CTG GTA CGT TTA TCA TCC CAG ACC GGG  1824
Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser Ser Gln Thr Gly
        595                 600                 605

CTA TCA TTT GAA GAA TTG GAC TGG CTG ATT GCC AAT GCC AGT CGT AGT  1872
Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn Ala Ser Arg Ser
    610                 615                 620

GTG CCG GAC CAC CAC GAC AAA ATT GTG CTG GAT AAG CCG GTC CTT GAA  1920
Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys Pro Val Leu Glu
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625                 630                 635                 640

GCA CTG GCA GAG TAT GTC AGC CTA AAA CAG CGC TAT GGG CTT GAT GCC 1968
Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr Gly Leu Asp Ala
                645                 650                 655

AAT ACC TTT GCG ACC TTC ATT AGT GCA GTA AAT CCT TAT ACG CCA GAT 2016
Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro Tyr Thr Pro Asp
            660                 665                 670

CAG ACA CCC AGT TTC TAT GAA ACC GCT TTC CGC TCT GCC GAC GGT AAT 2064
Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser Ala Asp Gly Asn
        675                 680                 685

CAT GTC ATT GCG CTA GGT ACA GAG GTG AAA TAT GCA GAA AAT GAG CAG 2112
His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala Glu Asn Glu Gln
    690                 695                 700

GAT GAG TTA GCC GCC ATA TGC TGC AAA GCA TTG GGT GTC ACC AGT GAT 2160
Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly Val Thr Ser Asp
705                 710                 715                 720

GAA CTG CTC CGT ATT GGT CGC TAT TGC TTC GGT AAT GCA GGC AGT TTT 2208
Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn Ala Gly Ser Phe
                725                 730                 735

ACC TTG GAT GAA TAT ACC GCC AGT CAG TTG TAT CGC TTC GGC GCC ATT 2256
Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg Phe Gly Ala Ile
            740                 745                 750

CCC CGT TTG TTT GGG CTG ACA TTT GCC CAA GCC GAA ATT TTA TGG CGT 2304
Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu Ile Leu Trp Arg
        755                 760                 765

CTG ATG GAA GGC GGA AAA GAT ATC TTA TTG CAA CAG TTA GGT CAG GCA 2352
Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln Leu Gly Gln Ala
    770                 775                 780

AAA TCC CTG CAA CCA CTG GCT ATT TTA CGC CGT ACC GAG CAG GTG CTG 2400
Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr Glu Gln Val Leu
785                 790                 795                 800

GAT TGG ATG TCG TCC GTA AAT CTA AGT CTG ACT TAT CTG CAA GGG ATG 2448
Asp Trp Met Ser Ser Val Asn Leu Ser Leu Thr Tyr Leu Gln Gly Met
                805                 810                 815

GTA AGT ACG CAA TGG AGC GGT ACC GCC ACC GCT GAG ATG TTC AAT TTC 2496
Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu Met Phe Asn Phe
            820                 825                 830

TTG GAA AAC GTT TGT GAC AGC GTG AAT AGT CAA GCT GCC ACT AAA GAA 2544
Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala Ala Thr Lys Glu
        835                 840                 845

ACA ATG GAT TCG GCG TTA CAG CAG AAA GTG CTG CGG GCG CTA AGC GCC 2592
Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg Ala Leu Ser Ala
    850                 855                 860

GGT TTC GGC ATT AAG AGC AAT GTG ATG GGT ATC GTC ACC TTC TGG CTG 2640
Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val Thr Phe Trp Leu
865                 870                 875                 880

GAG AAA ATC ACA ATC GGT AGT GAT AAT CCT TTT ACA TTG GCA AAC TAC 2688
Glu Lys Ile Thr Ile Gly Ser Asp Asn Pro Phe Thr Leu Ala Asn Tyr
                885                 890                 895
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TGG CAT GAT     CAA ACC CTG TTT AGC CAT GAC AAT GCC ACG TTA GAG  2736
Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn Ala Thr Leu Glu
            900                 905                 910

TCC TTA CAA ACC GAC ACT TCT CTG GTA ATT GCT ACT CAG CAA CTT AGC  2784
Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr Gln Gln Leu Ser
        915                 920                 925

CAG CTA GTG TTA ATT GTG AAA TGG CTG AGC CTG ACC GAG CAG GAT CTG  2832
Gln Leu Val Leu Ile Val Lys Trp Leu Ser Leu Thr Glu Gln Asp Leu
    930                 935                 940

CAA TTA CTG ACA ACC TAT CCC GAA CGT TTA ATC AAC GGC ATC ACG AAT  2880
Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn Gly Ile Thr Asn
945                 950                 955                 960

GTT CCT GTA CCC AAT CCG GAG CTA TTA CTC ACG CTA TCA CGT TTT AAG  2928
Val Pro Val Pro Asn Pro Glu Leu Leu Leu Thr Leu Ser Arg Phe Lys
                965                 970                 975

CAG TGG GAA ACT CAA GTC ACC GTT TCC CGT GAT GAA GCG ATG CGC TGT  2976
Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu Ala Met Arg Cys
            980                 985                 990

TTC GAT CAA TTA AAT GCC AAT GAT ATG ACG ACT GAA AAT GCA GGT TCA  3024
Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu Asn Ala Gly Ser
        995                 1000                1005

CTG ATC GCC ACA TTG TAT GAG ATG GAT AAA GGT ACG GGA GCG CAA GTT  3072
Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr Gly Ala Gln Val
    1010                1015                1020

AAT ACC TTG CTA TTA GGT GAA AAT AAC TGG CCG AAA AGT TTT ACC TCT  3120
Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys Ser Phe Thr Ser
1025                1030                1035                1040

CTC TGG CAA CTT CTG ACC TGG TTA CGC GTC GGG CAA AGA CTG AAT GTC  3168
Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln Arg Leu Asn Val
                1045                1050                1055

GGT AGT ACC ACT CTG GGC AAT CTG TTG TCC ATG ATG CAA GCA GAC CCT  3216
Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met Gln Ala Asp Pro
            1060                1065                1070

GCT GCC GAG AGT AGC GCT TTA TTG GCA TCA GTA GCC CAA AAC TTA AGT  3264
Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala Gln Asn Leu Ser
        1075                1080                1085

GCC GCA ATC AGC AAT CGT CAG TAA                                 3285
Ala Ala Ile Ser Asn Arg Gln •••
    1090                1095
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1095 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

| Features | From | To | Description |
|---|---|---|---|
| | 254 | 267 | SEQ ID NO:15 |
| | 254 | 492 | TcaAii peptide |

Met Val Thr Val Met Gln Asn Lys Ile Ser Phe Leu Ser Gly Thr Ser
1 5 10 15

Glu Gln Pro Leu Leu Asp Ala Gly Tyr Gln Asn Val Phe Asp Ile Ala
20 25 30

Ser Ile Ser Arg Ala Thr Phe Val Gln Ser Val Pro Thr Leu Pro Val
35 40 45

Lys Glu Ala His Thr Val Tyr Arg Gln Ala Arg Gln Arg Ala Glu Asn
50 55 60

Leu Lys Ser Leu Tyr Arg Ala Trp Gln Leu Arg Gln Glu Pro Val Ile
65 70 75 80

Lys Gly Leu Ala Lys Leu Asn Leu Gln Ser Asn Val Ser Val Leu Gln
85 90 95

Asp Ala Leu Val Glu Asn Ile Gly Gly Asp Gly Asp Phe Ser Asp Leu
100 105 110

Met Asn Arg Ala Ser Gln Tyr Ala Asp Ala Ala Ser Ile Gln Ser Leu
115 120 125

Phe Ser Pro Gly Arg Tyr Ala Ser Ala Leu Tyr Arg Val Ala Lys Asp
130 135 140

Leu His Lys Ser Asp Ser Ser Leu His Ile Asp Asn Arg Arg Ala Asp
145 150 155 160

Leu Lys Asp Leu Ile Leu Ser Glu Thr Thr Met Asn Lys Glu Val Thr
165 170 175

Ser Leu Asp Ile Leu Leu Asp Val Leu Gln Lys Gly Gly Lys Asp Ile
180 185 190

Thr Glu Leu Ser Gly Ala Phe Phe Pro Met Thr Leu Pro Tyr Asp Asp
195 200 205

His Leu Ser Gln Ile Asp Ser Ala Leu Ser Ala Gln Ala Arg Thr Leu
210 215 220

Asn Gly Val Trp Asn Thr Leu Thr Asp Thr Thr Ala Gln Ala Val Ser
225 230 235 240

Glu Gln Thr Ser Asn Thr Asn Thr Arg Lys Leu Phe Ala Ala Gln Asp
245 250 255

Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr Phe Tyr Phe Lys Ala
260 265 270

Val Gly Phe Ser Gly Gln Pro Met Val Tyr Leu Ser Gln Tyr Thr Ser
275 280 285

Gly Asn Gly Ile Val Gly Ala Gln Leu Ile Ala Gly Asn Pro Asp Gln
290 295 300

Ala Ala Ala Ala Ile Val Ala Pro Leu Lys Leu Thr Trp Ser Met Ala
305 310 315 320

Lys Gln Cys Tyr Tyr Leu Val Ala Pro Asp Gly Thr Thr Met Gly Asp
325 330 335

Gly Asn Val Leu Thr Gly Cys Phe Leu Arg Gly Asn Ser Pro Thr Asn

340 345 350

Pro Asp Lys Asp Gly Ile Phe Ala Gln Val Ala Asn Lys Ser Gly Ser
355 360 365

Thr Gln Pro Leu Pro Ser Phe His Leu Pro Val Thr Leu Glu His Ser
370 375 380

Glu Asn Lys Asp Gln Tyr Tyr Leu Lys Thr Glu Gln Gly Tyr Ile Thr
385 390 395 400

Val Asp Ser Ser Gly Gln Ser Asn Trp Lys Asn Ala Leu Val Ile Asn
405 410 415

Gly Thr Lys Asp Lys Gly Leu Leu Leu Thr Phe Cys Ser Asp Ser Ser
420 425 430

Gly Thr Pro Thr Asn Pro Asp Asp Val Ile Pro Pro Ala Ile Asn Asp
435 440 445

Ile Pro Ser Pro Pro Ala Arg Glu Thr Leu Ser Leu Thr Pro Val Ser
450 455 460

Tyr Gln Leu Met Thr Asn Pro Ala Pro Thr Glu Asp Asp Ile Thr Asn
465 470 475 480

His Tyr Gly Phe Asn Gly Ala Ser Leu Arg Ala Ser Pro Leu Ser Thr
485 490 W4 » 495

Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr Phe Cys Glu Lys
500 505 510

Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr Ala Gln Gln Ser
515 520 525

Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser Arg Tyr Val Arg
530 535 540

Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr Gly Ala Ala Tyr
545 550 555 560

Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln Tyr Leu Trp Ile
565 570 575

Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp Thr Val Val Ala
580 585 590

Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser Ser Gln Thr Gly
595 600 605

Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn Ala Ser Arg Ser
610 615 620

Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys Pro Val Leu Glu
625 630 635 640

Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr Gly Leu Asp Ala
645 650 655

Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro Tyr Thr Pro Asp
660 665 670

Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser Ala Asp Gly Asn
675 680 685

His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala Glu Asn Glu Gln

690 695 700

Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly Val Thr Ser Asp
705 710 715 720

Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn Ala Gly Ser Phe
725 730 735

Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg Phe Gly Ala Ile
740 745 750

Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu Ile Leu Trp Arg
755 760 765

Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln Leu Gly Gln Ala
770 775 780

Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr Glu Gln Val Leu
785 790 795 800

Asp Trp Met Ser Ser Val Asn Leu Ser Leu Thr Tyr Leu Gln Gly Met
805 810 815

Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu Met Phe Asn Phe
820 825 830

Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala Ala Thr Lys Glu
835 840 845

Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg Ala Leu Ser Ala
850 855 860

Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val Thr Phe Trp Leu
865 870 875 880

Glu Lys Ile Thr Ile Gly Ser Asp Asn Pro Phe Thr Leu Ala Asn Tyr
885 890 895

Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn Ala Thr Leu Glu
900 905 910

Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr Gln Gln Leu Ser
915 920 925

Gln Leu Val Leu Ile Val Lys Trp Leu Ser Leu Thr Glu Gln Asp Leu
930 935 940

Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn Gly Ile Thr Asn
945 950 955 960

Val Pro Val Pro Asn Pro Glu Leu Leu Leu Thr Leu Ser Arg Phe Lys
965 970 975

Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu Ala Met Arg Cys
980 985 990

Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu Asn Ala Gly Ser
995 1000 1005

Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr Gly Ala Gln Val
1010 1015 1020

Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys Ser Phe Thr Ser
1025 1030 1035 1040

Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln Arg Leu Asn Val

1045 1050 1055

Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met Gln Ala Asp Pro
1060 1065 1070

Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala Gln Asn Leu Ser
1075 1080 1085

Ala Ala Ile Ser Asn Arg Gln •••
1090 1095

(2) INFORMATION FOR SEQ ID NO:35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 603 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Pro Leu Ser Thr Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr
1 5 10 15

Phe Cys Glu Lys Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr
20 25 30

Ala Gln Gln Ser Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser
35 40 45

Arg Tyr Val Arg Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr
50 55 60

Gly Ala Ala Tyr Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln
65 70 75 80

Tyr Leu Trp Ile Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp
85 90 95

Thr Val Val Ala Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser
100 105 110

Ser Gln Thr Gly Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn
115 120 125

Ala Ser Arg Ser Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys
130 135 140

Pro Val Leu Glu Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr
145 150 155 160

Gly Leu Asp Ala Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro
165 170 175

Tyr Thr Pro Asp Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser
180 185 190

Ala Asp Gly Asn His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala
195 200 205

Glu Asn Glu Gln Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly
210 215 220

Val Thr Ser Asp Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn
225 230 235 240

Ala Gly Arg Phe Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg
245 250 255

Phe Gly Ala Ile Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu
260 265 270

Ile Leu Trp Arg Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln
275 280 285

Xxx Gly Gln Ala Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr
290 295 300

Glu Gln Val Leu Asp Trp Met Ser Pro Val Asn Leu Ser Leu Thr Tyr
305 310 315 320

Leu Gln Gly Met Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu
325 330 335

Met Phe Asn Phe Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala
340 345 350

Xxx Thr Lys Glu Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg
355 360 365

Ala Leu Ser Ala Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val
370 375 380

Thr Phe Trp Leu Glu Lys Ile Thr Ile Gly Arg Asp Asn Pro Phe Thr
385 390 395 400

Leu Ala Asn Tyr Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn
405 410 415

Ala Thr Leu Glu Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr
420 425 430

Gln Gln Leu Ser Gln Leu Val Leu Ile Val Lys Trp Val Ser Leu Thr
435 440 445

Glu Gln Asp Leu Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn
450 455 460

Gly Ile Thr Asn Val Pro Val Pro Asn Pro Glu Leu Leu Leu Thr Leu
465 470 475 480

Ser Arg Phe Lys Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu
485 490 495

Ala Met Arg Cys Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu
500 505 510

Asn Ala Gly Ser Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr
515 520 525

Gly Ala Gln Val Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys
530 535 540

Ser Phe Thr Ser Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln
545 550 555 560

Arg Leu Asn Val Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met
565 570 575

Gln Ala Asp Pro Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala
580 585 590

Gln Asn Leu Ser Ala Ala Ile Ser Asn Arg Gln *
595 600

(2) INFORMATION FOR SEQ ID NO:36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2557 base pairs
(B) TYPE: nucleic acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GAATTCGGCT TGCGTTTAAT ATTGATGATG TCTCGCTCTT CCGCCTGCTT AAAATTACCG 60
ACCATGATAA TAAAGATGGA AAAATTAAAA ATAACCTAAA GAATCTTTCC AATTTATATA 120
TTGGAAAATT ACTGGCAGAT ATTCATCAAT TAACCATTGA TGAACTGGAT TTATTACTGA 180
TTGCCGTAGG TGAAGGAAAA ACTAATTTAT CCGCTATCAG TGATAAGCAA TTGGCTACCC 240
TGATCAGAAA ACTCAATACT ATTACCAGCT GGCTACATAC ACAGAAGTGG AGTGTATTCC 300
AGCTATTTAT CATGACCTCC ACCAGCTATA ACAAAACGCT AACGCCTGAA ATTAAGAATT 360
TGCTGGATAC CGTCTACCAC GGTTTACAAG GTTTTGATAA AGACAAAGCA GATTTGCTAC 420
ATGTCATGGC GCCCTATATT GCGGCCACCT TGCAATTATC ATCGGAAAAT GTCGCCCACT 480
CGGTACTCCT TTGGGCAGAT AAGTTACAGC CCGGCGACGG CGCAATGACA GCAGAGGGAN 540
TCTGGGACTG GTTGAATACT AAGTATACGC CGGGTTCATC GGAAGCCGTA GAAACGCAGG 600
AACATATCGT TCAGTATTGT CAGGCTCTGG CACAATTGGA AATGGTTTAC CATTCCACCG 660
GCATCAACGA AAACGCCTTC CGTCTATTTG TGACAAAACC AGAGATGTTT GGCGCTGCAA 720
CTGGAGCAGC GCCCGCGCAT GATGCCCTTT CACTGATTAT GCTGACACGT TTTGCGGATT 780
GGGTGAACGC ACTAGGCGAA AAAGCGTCCT CGGTGCTAGC GGCATTTGAA GCTAACTCGT 840
TAACGGCAGA ACAACTGGCT GATGCCATGA ATCTTGATGC TAATTTGCTG TTGCAAGCCA 900
GTATTCAAGC ACAAAATCAT CAACATCTTC CCCCAGTAAC TCCAGAAAAT GCGTTCTCCT 960
GTTGGACATC TATCAATACT ATCCTGCAAT GGGTTAATGT CGCACAACAA TTGAAATGTC 1020
GCCCCACAGG GCGTTTCCGC TTTGGTCGGG CTGGATTATA TTCAATCAAT GAAAGAGACA 1080
CCGACCTATG CCCAGTGGGA AAACGCGGCA GGCGTATTAA CCGCCGGGTT GAATTCAACA 1140
ACAGGCTAAT ACATTACAAC GCTTTTCTGG ATGAATCTCG CAGTGCCGCA TTAAGCACCT 1200
ACTATATCCG TCAAGTCGCC AAGGCAGCGG CGGCTATTAA AAGCCGTGAT GACTTGTATC 1260
AATACTTACT GATTGATAAT CAGGTTTCTG CGGCAATAAA AACCACCCGG ATCGCCGAAG 1320
CCATTGCCAG TATTCAACTG TACGTCAACC GGGCATTGGA AAATGTGGAA GAAAATGCCA 1380
ATTCGGGGGT TATCAGCCGC CAATTCTTTA TCGACTGGGA CAAATACAAT AAACGCTACA 1440
GCACTTGGGC GGGTGTTTCT CAATTAGTTT ACTACCCGGA AAACTATATT GATCCGACCA 1500
TGCGTATCGG ACAAACCAAA ATGATGGACG CATTACTGCA ATCCGTCAGC CAAAGCCAAT 1560
TAAACGCCGA TACCGTCGAA GATGCCTTTA TGTCTTATCT GACATCGTTT GAACAAGTGG 1620
CTAATCTTAA AGTTATTAGC GCATATCACG ATAATATTAA TAACGATCAA GGGCTGACCT 1680
ATTTTATCGG ACTCAGTGAA ACTGATGCCG GTGAATATTA TTGGCGCAGT GTCGATCACA 1740
GTAAATTCAA CGACGGTAAA TTCGCGGCTA ATGCCTGGAG TGAATGGCAT AAAATTGATT 1800
GTCCAATTAA CCCTTATAAA AGCACTATCC GTCCAGTGAT ATATAAATCC CGCCTGTATC 1860

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TGCTCTGGTT GGAACAAAAG GAGATCACCA AACAGACAGG AAATAGTAAA GATGGCTATC  1920
AAACTGAAAC GGATTATCGT TATGAACTAA AATTGGCGCA TATCCGCTAT GATGGCACTT  1980
GGAATACGCC AATCACCTTT GATGTCAATA AAAAAATATC CGAGCTAAAA CTGGAAAAAA  2040
ATAGAGCGCC CGGACTCTAT TGTGCCGGTT ATCAAGGTGA AGATACGTTG CTGGTGATGT  2100
TTTATAACCA ACAAGACACA CTAGATAGTT ATAAAAACGC TTCAATGCAA GGACTATATA  2160
TCTTTGCTGA TATGGCATCC AAAGATATGA CCCCAGAACA GAGCAATGTT TATCGGGATA  2220
ATAGCTATCA ACAATTTGAT ACCAATAATG TCAGAAGAGT GAATAACCGC TATGCAGAGG  2280
ATTATGAGAT TCCTTCTTCG GTAAGTAGCC GTAAAGACTA TGGTTGGGGA GATTATTACC  2340
TCAGCATGGT ATATAACGGA GATATTCCAA CTATCAATTA CAAAGCCGCA TCAAGTGATT  2400
TAAAAATTTA TATTTCACCA AAATTAAGAA TTATTCATAA TGGATATGAA GGACAGAAGC  2460
GCAATCAATG CAATTTGATG AATAAATATG GCAAACTAGG TGATAAATTT ATTGTGTATA  2520
CCAGCCTGGG CGTTAATCCG AATAATAAGC CGAATTC                           2557
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(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 845 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein (partial)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Ala Phe Asn Ile Asp Asp Val Ser Leu Phe Arg Leu Leu Lys Ile Thr
 1               5                  10                  15

Asp His Asp Asn Lys Asp Gly Lys Ile Lys Asn Asn Leu Lys Asn Leu
            20                  25                  30

Ser Asn Leu Tyr Ile Gly Lys Leu Leu Ala Asp Ile His Gln Leu Thr
        35                  40                  45

Ile Asp Glu Leu Asp Leu Leu Leu Ile Ala Val Gly Glu Gly Lys Thr
    50                  55                  60

Asn Leu Ser Ala Ile Ser Asp Lys Gln Leu Ala Thr Leu Ile Arg Lys
65                  70                  75                  80

Leu Asn Thr Ile Thr Ser Trp Leu His Thr Gln Lys Trp Ser Val Phe
                85                  90                  95

Gln Leu Phe Ile Met Thr Ser Thr Ser Tyr Asn Lys Thr Leu Thr Pro
            100                 105                 110

Glu Ile Lys Asn Leu Leu Asp Thr Val Tyr His Gly Leu Gln Gly Phe
        115                 120                 125

Asp Lys Asp Lys Ala Asp Leu Leu His Val Met Ala Pro Tyr Ile Ala
    130                 135                 140

Ala Thr Leu Gln Leu Ser Ser Glu Asn Val Ala His Ser Val Leu Leu
145                 150                 155                 160

Trp Ala Asp Lys Leu Gln Pro Gly Asp Gly Ala Met Thr Ala Glu Gly
                165                 170                 175

Phe Trp Asp Trp Leu Asn Thr Lys Tyr Thr Pro Gly Ser Ser Glu Ala
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180 185 190

Val Glu Thr Gln Glu His Ile Val Gln Tyr Cys Gln Ala Leu Ala Gln
195 200 205

Leu Glu Met Val Tyr His Ser Thr Gly Ile Asn Glu Asn Ala Phe Arg
210 215 220

Leu Phe Val Thr Lys Pro Glu Met Phe Gly Ala Ala Thr Gly Ala Ala
225 230 235 240

Pro Ala His Asp Ala Leu Ser Leu Ile Met Leu Thr Arg Phe Ala Asp
245 250 255

Trp Val Asn Ala Leu Gly Glu Lys Ala Ser Ser Val Leu Ala Ala Phe
260 265 270

Glu Ala Asn Ser Leu Thr Ala Glu Gln Leu Ala Asp Ala Met Asn Leu
275 280 285

Asp Ala Asn Leu Leu Leu Gln Ala Ser Ile Gln Ala Gln Asn His Gln
290 295 300

His Leu Pro Pro Val Thr Pro Glu Asn Ala Phe Ser Cys Trp Thr Ser
305 310 315 320

Ile Asn Thr Ile Leu Gln Trp Val Asn Val Ala Gln Gln Leu Lys Cys
325 330 335

Arg Pro Thr Gly Arg Phe Arg Phe Gly Arg Ala Gly Leu Tyr Ser Ile
340 345 350

Asn Glu Arg Asp Thr Asp Leu Cys Pro Val Gly Lys Arg Gly Arg Arg
355 360 365

Ile Asn Arg Arg Val Glu Phe Asn Asn Arg Leu Ile His Tyr Asn Ala
370 375 380

Phe Leu Asp Glu Ser Arg Ser Ala Ala Leu Ser Thr Tyr Tyr Ile Arg
385 390 395 400

Gln Val Ala Lys Ala Ala Ala Ala Ile Lys Ser Arg Asp Asp Leu Tyr
405 410 415

Gln Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Ala Ile Lys Thr Thr
420 425 430

Arg Ile Ala Glu Ala Ile Ala Ser Ile Gln Leu Tyr Val Asn Arg Ala
435 440 445

Leu Glu Asn Val Glu Glu Asn Ala Asn Ser Gly Val Ile Ser Arg Gln
450 455 460

Phe Phe Ile Asp Trp Asp Lys Tyr Asn Lys Arg Tyr Ser Thr Trp Ala
465 470 475 480

Gly Val Ser Gln Leu Val Tyr Tyr Pro Glu Asn Tyr Ile Asp Pro Thr
485 490 495

Met Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln Ser Val
500 505 510

Ser Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe Met Ser
515 520 525

Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile Ser Ala

530 535 540

Tyr His Asp Asn Ile Asn Asn Asp Gln Gly Leu Thr Tyr Phe Ile Gly
545 550 555 560

Leu Ser Glu Thr Asp Ala Gly Glu Tyr Tyr Trp Arg Ser Val Asp His
565 570 575

Ser Lys Phe Asn Asp Gly Lys Phe Ala Ala Asn Ala Trp Ser Glu Trp
580 585 590

His Lys Ile Asp Cys Pro Ile Asn Pro Tyr Lys Ser Thr Ile Arg Pro
595 600 605

Val Ile Tyr Lys Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln Lys Glu
610 615 620

Ile Thr Lys Gln Thr Gly Asn Ser Lys Asp Gly Tyr Gln Thr Glu Thr
625 630 635 640

Asp Tyr Arg Tyr Glu Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Thr
645 650 655

Trp Asn Thr Pro Ile Thr Phe Asp Val Asn Lys Lys Ile Ser Glu Leu
660 665 670

Lys Leu Glu Lys Asn Arg Ala Pro Gly Leu Tyr Cys Ala Gly Tyr Gln
675 680 685

Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Asn Gln Gln Asp Thr Leu
690 695 700

Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp
705 710 715 720

Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp
725 730 735

Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn
740 745 750

Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys
755 760 765

Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp
770 775 780

Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr
785 790 795 800

Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys
805 810 815

Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys
820 825 830

Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn
835 840 845

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid

(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly
1 5 10 15

Lys

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala
1 5 10 15

Ile Ser Pro Ala Lys
20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ala Asn Ser Leu Tyr Ala Leu Phe Leu Pro Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids

(B) TYPE: amino acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Glu Val Tyr
1 5 10 15

Ala Gly Leu Glu

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ile Arg Glu Asp Tyr Pro Ala Ser Leu Gly Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
Asp Asp Ser Gly Asp Asp Asp Lys Val Thr Asn Thr Asp Ile His
1               5                  10                  15

Arg
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
Asp Val Xaa Gly Ser Glu Lys Ala Asn Glu Lys Leu Lys
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7551 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46 (*ccdA*):

```
ATG AAC GAG TCT GTA AAA GAG ATA CCT GAT GTA TTA AAA AGC CAG TGT  48
Met Asn Glu Ser Val Lys Glu Ile Pro Asp Val Leu Lys Ser Gln Cys
 1               5                  10                  15

GGT TTT AAT TGT CTG ACA GAT ATT AGC CAC AGC TCT TTT AAT GAA TTT  96
Gly Phe Asn Cys Leu Thr Asp Ile Ser His Ser Ser Phe Asn Glu Phe
            20                  25                  30

CGC CAG CAA GTA TCT GAG CAC CTC TCC TGG TCC GAA ACA CAC GAC TTA 144
Arg Gln Gln Val Ser Glu His Leu Ser Trp Ser Glu Thr His Asp Leu
        35                  40                  45

TAT CAT GAT GCA CAA CAG GCA CAA AAG GAT AAT CGC CTG TAT GAA GCG 192
Tyr His Asp Ala Gln Gln Ala Gln Lys Asp Asn Arg Leu Tyr Glu Ala
    50                  55                  60

CGT ATT CTC AAA CGC GCC AAT CCC CAA TTA CAA AAT GCG GTG CAT CTT 240
Arg Ile Leu Lys Arg Ala Asn Pro Gln Leu Gln Asn Ala Val His Leu
65                  70                  75                  80
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GCC ATT     GCT CCC AAT GCT GAA CTG ATA GGC TAT AAC AAT CAA TTT  288
Ala Ile Leu Ala Pro Asn Ala Glu Leu Ile Gly Tyr Asn Asn Gln Phe
                85                  90                  95

AGC GGT AGA GCC AGT CAA TAT GTT GCG CCG GGT ACC GTT TCT TCC ATG  336
Ser Gly Arg Ala Ser Gln Tyr Val Ala Pro Gly Thr Val Ser Ser Met
            100                 105                 110

TTC TCC CCC GCC GCT TAT TTG ACT GAA CTT TAT CGT GAA GCA CGC AAT  384
Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Arg Asn
        115                 120                 125

TTA CAC GCA AGT GAC TCC GTT TAT TAT CTG GAT ACC CGC CGC CCA GAT  432
Leu His Ala Ser Asp Ser Val Tyr Tyr Leu Asp Thr Arg Arg Pro Asp
    130                 135                 140

CTC AAA TCA ATG GCG CTC AGT CAG CAA AAT ATG GAT ATA GAA TTA TCC  480
Leu Lys Ser Met Ala Leu Ser Gln Gln Asn Met Asp Ile Glu Leu Ser
145                 150                 155                 160

ACA CTC TCT TTG TCC AAT GAG CTG TTA TTG GAA AGC ATT AAA ACT GAA  528
Thr Leu Ser Leu Ser Asn Glu Leu Leu Leu Glu Ser Ile Lys Thr Glu
                165                 170                 175

TCT AAA CTG GAA AAC TAT ACT AAA GTG ATG GAA ATG CTC TCC ACT TTC  576
Ser Lys Leu Glu Asn Tyr Thr Lys Val Met Glu Met Leu Ser Thr Phe
            180                 185                 190

CGT CCT TCC GGC GCA ACG CCT TAT CAT GAT GCT TAT GAA AAT GTG CGT  624
Arg Pro Ser Gly Ala Thr Pro Tyr His Asp Ala Tyr Glu Asn Val Arg
        195                 200                 205

GAA GTT ATC CAG CTA CAA GAT CCT GGA CTT GAG CAA CTC AAT GCA TCA  672
Glu Val Ile Gln Leu Gln Asp Pro Gly Leu Glu Gln Leu Asn Ala Ser
    210                 215                 220

CCG GCA ATT GCC GGG TTG ATG CAT CAA GCC TCC CTA TTG GGT ATT AAC  720
Pro Ala Ile Ala Gly Leu Met His Gln Ala Ser Leu Leu Gly Ile Asn
225                 230                 235                 240

GCT TCA ATC TCG CCT GAG CTA TTT AAT ATT CTG ACG GAG GAG ATT ACC  768
Ala Ser Ile Ser Pro Glu Leu Phe Asn Ile Leu Thr Glu Glu Ile Thr
                245                 250                 255

GAA GGT AAT GCT GAG GAA CTT TAT AAG AAA AAT TTT GGT AAT ATC GAA  816
Glu Gly Asn Ala Glu Glu Leu Tyr Lys Lys Asn Phe Gly Asn Ile Glu
            260                 265                 270

CCG GCC TCA TTG GCT ATG CCG GAA TAC CTT AAA CGT TAT TAT AAT TTA  864
Pro Ala Ser Leu Ala Met Pro Glu Tyr Leu Lys Arg Tyr Tyr Asn Leu
        275                 280                 285

AGC GAT GAA GAA CTT AGT CAG TTT ATT GGT AAA GCC AGC AAT TTT GGT  912
Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly Lys Ala Ser Asn Phe Gly
    290                 295                 300

CAA CAG GAA TAT AGT AAT AAC CAA CTT ATT ACT CCG GTA GTC AAC AGC  960
Gln Gln Glu Tyr Ser Asn Asn Gln Leu Ile Thr Pro Val Val Asn Ser
305                 310                 315                 320

AGT GAT GGC ACG GTT AAG GTA TAT CGG ATC ACC CGC GAA TAT ACA ACC 1008
Ser Asp Gly Thr Val Lys Val Tyr Arg Ile Thr Arg Glu Tyr Thr Thr
                325                 330                 335

AAT GCT TAT CAA ATG GAT GTG GAG CTA TTT CCC TTC GGT GGT GAG AAT 1056
Asn Ala Tyr Gln Met Asp Val Glu Leu Phe Pro Phe Gly Gly Glu Asn
            340                 345                 350

TAT CGG TTA GAT TAT AAA TTC AAA AAT TTT TAT AAT GCC TCT TAT TTA 1104
Tyr Arg Leu Asp Tyr Lys Phe Lys Asn Phe Tyr Asn Ala Ser Tyr Leu
        355                 360                 365
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TCC ATC AAG TTA AAT GAT AAA AGA GAA CTT GTT CGA ACT GAA GGC GCT 1152
Ser Ile Lys Leu Asn Asp Lys Arg Glu Leu Val Arg Thr Glu Gly Ala
    370                 375                 380

CCT CAA GTC AAT ATA GAA TAC TCC GCA AAT ATC ACA TTA AAT ACC GCT 1200
Pro Gln Val Asn Ile Glu Tyr Ser Ala Asn Ile Thr Leu Asn Thr Ala
385                 390                 395                 400

GAT ATC AGT CAA CCT TTT GAA ATT GGC CTG ACA CGA GTA CTT CCT TCC 1248
Asp Ile Ser Gln Pro Phe Glu Ile Gly Leu Thr Arg Val Leu Pro Ser
                405                 410                 415

GGT TCT TGG GCA TAT GCC GCC GCA AAA TTT ACC GTT GAA GAG TAT AAC 1296
Gly Ser Trp Ala Tyr Ala Ala Ala Lys Phe Thr Val Glu Glu Tyr Asn
            420                 425                 430

CAA TAC TCT TTT CTG CTA AAA CTT AAC AAG GCT ATT CGT CTA TCA CGT 1344
Gln Tyr Ser Phe Leu Leu Lys Leu Asn Lys Ala Ile Arg Leu Ser Arg
        435                 440                 445

GCG ACA GAA TTG TCA CCC ACG ATT CTG GAA GGC ATT GTG CGC AGT GTT 1392
Ala Thr Glu Leu Ser Pro Thr Ile Leu Glu Gly Ile Val Arg Ser Val
    450                 455                 460

AAT CTA CAA CTG GAT ATC AAC ACA GAC GTA TTA GGT AAA GTT TTT CTG 1440
Asn Leu Gln Leu Asp Ile Asn Thr Asp Val Leu Gly Lys Val Phe Leu
465                 470                 475                 480

ACT AAA TAT TAT ATG CAG CGT TAT GCT ATT CAT GCT GAA ACT GCC CTG 1488
Thr Lys Tyr Tyr Met Gln Arg Tyr Ala Ile His Ala Glu Thr Ala Leu
                485                 490                 495

ATA CTA TGC AAC GCG CCT ATT TCA CAA CGT TCA TAT GAT AAT CAA CCT 1536
Ile Leu Cys Asn Ala Pro Ile Ser Gln Arg Ser Tyr Asp Asn Gln Pro
            500                 505                 510

AGC CAA TTT GAT CGC CTG TTT AAT ACG CCA TTA CTG AAC GGA CAA TAT 1584
Ser Gln Phe Asp Arg Leu Phe Asn Thr Pro Leu Leu Asn Gly Gln Tyr
        515                 520                 525

TTT TCT ACC GGC GAT GAG GAG ATT GAT TTA AAT TCA GGT AGC ACC GGC 1632
Phe Ser Thr Gly Asp Glu Glu Ile Asp Leu Asn Ser Gly Ser Thr Gly
    530                 535                 540

GAT TGG CGA AAA ACC ATA CTT AAG CGT GCA TTT AAT ATT GAT GAT GTC 1680
Asp Trp Arg Lys Thr Ile Leu Lys Arg Ala Phe Asn Ile Asp Asp Val
545                 550                 555                 560

TCG CTC TTC CGC CTG CTT AAA ATT ACC GAC CAT GAT AAT AAA GAT GGA 1728
Ser Leu Phe Arg Leu Leu Lys Ile Thr Asp His Asp Asn Lys Asp Gly
                565                 570                 575

AAA ATT AAA AAT AAC CTA AAG AAT CTT TCC AAT TTA TAT ATT GGA AAA 1776
Lys Ile Lys Asn Asn Leu Lys Asn Leu Ser Asn Leu Tyr Ile Gly Lys
            580                 585                 590

TTA CTG GCA GAT ATT CAT CAA TTA ACC ATT GAT GAA CTG GAT TTA TTA 1824
Leu Leu Ala Asp Ile His Gln Leu Thr Ile Asp Glu Leu Asp Leu Leu
        595                 600                 605

CTG ATT GCC GTA GGT GAA GGA AAA ACT AAT TTA TCC GCT ATC AGT GAT 1872
Leu Ile Ala Val Gly Glu Gly Lys Thr Asn Leu Ser Ala Ile Ser Asp
    610                 615                 620

AAG CAA TTG GCT ACC CTG ATC AGA AAA CTC AAT ACT ATT ACC AGC TGG 1920
Lys Gln Leu Ala Thr Leu Ile Arg Lys Leu Asn Thr Ile Thr Ser Trp
625                 630                 635                 640

CTA CAT ACA CAG AAG TGG AGT GTA TTC CAG CTA TTT ATC ATG ACC TCC 1968
Leu His Thr Gln Lys Trp Ser Val Phe Gln Leu Phe Ile Met Thr Ser
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                                645                 650                 655

ACC AGC TAT AAC AAA ACG CTA ACG CCT GAA ATT AAG AAT TTG CTG GAT  2016
Thr Ser Tyr Asn Lys Thr Leu Thr Pro Glu Ile Lys Asn Leu Leu Asp
                660                 665                 670

ACC GTC TAC CAC GGT TTA CAA GGT TTT GAT AAA GAC AAA GCA GAT TTG  2064
Thr Val Tyr His Gly Leu Gln Gly Phe Asp Lys Asp Lys Ala Asp Leu
            675                 680                 685

CTA CAT GTC ATG GCG CCC TAT ATT GCG GCC ACC TTG CAA TTA TCA TCG  2112
Leu His Val Met Ala Pro Tyr Ile Ala Ala Thr Leu Gln Leu Ser Ser
        690                 695                 700

GAA AAT GTC GCC CAC TCG GTA CTC CTT TGG GCA GAT AAG TTA CAG CCC  2160
Glu Asn Val Ala His Ser Val Leu Leu Trp Ala Asp Lys Leu Gln Pro
705                 710                 715                 720

GGC GAC GGC GCA ATG ACA GCA GAA AAA TTC TGG GAC TGG TTG AAT ACT  2208
Gly Asp Gly Ala Met Thr Ala Glu Lys Phe Trp Asp Trp Leu Asn Thr
                725                 730                 735

AAG TAT ACG CCG GGT TCA TCG GAA GCC GTA GAA ACG CAG GAA CAT ATC  2256
Lys Tyr Thr Pro Gly Ser Ser Glu Ala Val Glu Thr Gln Glu His Ile
            740                 745                 750

GTT CAG TAT TGT CAG GCT CTG GCA CAA TTG GAA ATG GTT TAC CAT TCC  2304
Val Gln Tyr Cys Gln Ala Leu Ala Gln Leu Glu Met Val Tyr His Ser
        755                 760                 765

ACC GGC ATC AAC GAA AAC GCC TTC CGT CTA TTT GTG ACA AAA CCA GAG  2352
Thr Gly Ile Asn Glu Asn Ala Phe Arg Leu Phe Val Thr Lys Pro Glu
    770                 775                 780

ATG TTT GGC GCT GCA ACT GGA GCA GCG CCC GCG CAT GAT GCC CTT TCA  2400
Met Phe Gly Ala Ala Thr Gly Ala Ala Pro Ala His Asp Ala Leu Ser
785                 790                 795                 800

CTG ATT ATG CTG ACA CGT TTT GCG GAT TGG GTG AAC GCA CTA GGC GAA  2448
Leu Ile Met Leu Thr Arg Phe Ala Asp Trp Val Asn Ala Leu Gly Glu
                805                 810                 815

AAA GCG TCC TCG GTG CTA GCG GCA TTT GAA GCT AAC TCG TTA ACG GCA  2496
Lys Ala Ser Ser Val Leu Ala Ala Phe Glu Ala Asn Ser Leu Thr Ala
            820                 825                 830

GAA CAA CTG GCT GAT GCC ATG AAT CTT GAT GCT AAT TTG CTG TTG CAA  2544
Glu Gln Leu Ala Asp Ala Met Asn Leu Asp Ala Asn Leu Leu Leu Gln
        835                 840                 845

GCC AGT ATT CAA GCA CAA AAT CAT CAA CAT CTT CCC CCA GTA ACT CCA  2592
Ala Ser Ile Gln Ala Gln Asn His Gln His Leu Pro Pro Val Thr Pro
    850                 855                 860

GAA AAT GCG TTC TCC TGT TGG ACA TCT ATC AAT ACT ATC CTG CAA TGG  2640
Glu Asn Ala Phe Ser Cys Trp Thr Ser Ile Asn Thr Ile Leu Gln Trp
865                 870                 875                 880

GTT AAT GTC GCA CAA CAA TTG AAT GTC GCC CCA CAG GGC GTT TCC GCT  2688
Val Asn Val Ala Gln Gln Leu Asn Val Ala Pro Gln Gly Val Ser Ala
                885                 890                 895

TTG GTC GGG CTG GAT TAT ATT CAA TCA ATG AAA GAG ACA CCG ACC TAT  2736
Leu Val Gly Leu Asp Tyr Ile Gln Ser Met Lys Glu Thr Pro Thr Tyr
            900                 905                 910

GCC CAG TGG GAA AAC GCG GCA GGC GTA TTA ACC GCC GGG TTG AAT TCA  2784
Ala Gln Trp Glu Asn Ala Ala Gly Val Leu Thr Ala Gly Leu Asn Ser
        915                 920                 925

CAA CAG GCT AAT ACA TTA CAC GCT TTT CTG GAT GAA TCT CGC AGT GCC  2832
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Gln Gln Ala Asn Thr Leu His Ala Phe Leu Asp Glu Ser Arg Ser Ala
     930                 935                 940

GCA TTA AGC ACC TAC TAT ATC CGT CAA GTC GCC AAG GCA GCG GCG GCT  2880
Ala Leu Ser Thr Tyr Tyr Ile Arg Gln Val Ala Lys Ala Ala Ala Ala
945                 950                 955                 960

ATT AAA AGC CGT GAT GAC TTG TAT CAA TAC TTA CTG ATT GAT AAT CAG  2928
Ile Lys Ser Arg Asp Asp Leu Tyr Gln Tyr Leu Leu Ile Asp Asn Gln
                965                 970                 975

GTT TCT GCG GCA ATA AAA ACC ACC CGG ATC GCC GAA GCC ATT GCC AGT  2976
Val Ser Ala Ala Ile Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Ser
            980                 985                 990

ATT CAA CTG TAC GTC AAC CGG GCA TTG GAA AAT GTG GAA GAA AAT GCC  3024
Ile Gln Leu Tyr Val Asn Arg Ala Leu Glu Asn Val Glu Glu Asn Ala
        995                 1000                1005

AAT TCG GGG GTT ATC AGC CGC CAA TTC TTT ATC GAC TGG GAC AAA TAC  3072
Asn Ser Gly Val Ile Ser Arg Gln Phe Phe Ile Asp Trp Asp Lys Tyr
    1010                1015                1020

AAT AAA CGC TAC AGC ACT TGG GCG GGT GTT TCT CAA TTA GTT TAC TAC  3120
Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Gln Leu Val Tyr Tyr
1025                1030                1035                1040

CCG GAA AAC TAT ATT GAT CCG ACC ATG CGT ATC GGA CAA ACC AAA ATG  3168
Pro Glu Asn Tyr Ile Asp Pro Thr Met Arg Ile Gly Gln Thr Lys Met
                1045                1050                1055

ATG GAC GCA TTA CTG CAA TCC GTC AGC CAA AGC CAA TTA AAC GCC GAT  3216
Met Asp Ala Leu Leu Gln Ser Val Ser Gln Ser Gln Leu Asn Ala Asp
            1060                1065                1070

ACC GTC GAA GAT GCC TTT ATG TCT TAT CTG ACA TCG TTT GAA CAA GTG  3264
Thr Val Glu Asp Ala Phe Met Ser Tyr Leu Thr Ser Phe Glu Gln Val
        1075                1080                1085

GCT AAT CTT AAA GTT ATT AGC GCA TAT CAC GAT AAT ATT AAT AAC GAT  3312
Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Ile Asn Asn Asp
    1090                1095                1100

CAA GGG CTG ACC TAT TTT ATC GGA CTC AGT GAA ACT GAT GCC GGT GAA  3360
Gln Gly Leu Thr Tyr Phe Ile Gly Leu Ser Glu Thr Asp Ala Gly Glu
1105                1110                1115                1120

TAT TAT TGG CGC AGT GTC GAT CAC AGT AAA TTC AAC GAC GGT AAA TTC  3408
Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Phe Asn Asp Gly Lys Phe
                1125                1130                1135

GCG GCT AAT GCC TGG AGT GAA TGG CAT AAA ATT GAT TGT CCA ATT AAC  3456
Ala Ala Asn Ala Trp Ser Glu Trp His Lys Ile Asp Cys Pro Ile Asn
            1140                1145                1150

CCT TAT AAA AGC ACT ATC CGT CCA GTG ATA TAT AAA TCC CGC CTG TAT  3504
Pro Tyr Lys Ser Thr Ile Arg Pro Val Ile Tyr Lys Ser Arg Leu Tyr
        1155                1160                1165

CTG CTC TGG TTG GAA CAA AAG GAG ATC ACC AAA CAG ACA GGA AAT AGT  3552
Leu Leu Trp Leu Glu Gln Lys Glu Ile Thr Lys Gln Thr Gly Asn Ser
    1170                1175                1180

AAA GAT GGC TAT CAA ACT GAA ACG GAT TAT CGT TAT GAA CTA AAA TTG  3600
Lys Asp Gly Tyr Gln Thr Glu Thr Asp Tyr Arg Tyr Glu Leu Lys Leu
1185                1190                1195                1200

GCG CAT ATC CGC TAT GAT GGC ACT TGG AAT ACG CCA ATC ACC TTT GAT  3648
Ala His Ile Arg Tyr Asp Gly Thr Trp Asn Thr Pro Ile Thr Phe Asp
                1205                1210                1215
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GTC AAT AAA AAA ATA TCC GAG CTA AAA CTG GAA AAA AAT AGA GCG CCC  3696
Val Asn Lys Lys Ile Ser Glu Leu Lys Leu Glu Lys Asn Arg Ala Pro
          1220                1225                1230

GGA CTC TAT TGT GCC GGT TAT CAA GGT GAA GAT ACG TTG CTG GTG ATG  3744
Gly Leu Tyr Cys Ala Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met
     1235                1240                1245

TTT TAT AAC CAA CAA GAC ACA CTA GAT AGT TAT AAA AAC GCT TCA ATG  3792
Phe Tyr Asn Gln Gln Asp Thr Leu Asp Ser Tyr Lys Asn Ala Ser Met
1250                1255                1260

CAA GGA CTA TAT ATC TTT GCT GAT ATG GCA TCC AAA GAT ATG ACC CCA  3840
Gln Gly Leu Tyr Ile Phe Ala Asp Met Ala Ser Lys Asp Met Thr Pro
1265                1270                1275                1280

GAA CAG AGC AAT GTT TAT CGG GAT AAT AGC TAT CAA CAA TTT GAT ACC  3888
Glu Gln Ser Asn Val Tyr Arg Asp Asn Ser Tyr Gln Gln Phe Asp Thr
               1285                1290                1295

AAT AAT GTC AGA AGA GTG AAT AAC CGC TAT GCA GAG GAT TAT GAG ATT  3936
Asn Asn Val Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr Glu Ile
          1300                1305                1310

CCT TCC TCG GTA AGT AGC CGT AAA GAC TAT GGT TGG GGA GAT TAT TAC  3984
Pro Ser Ser Val Ser Ser Arg Lys Asp Tyr Gly Trp Gly Asp Tyr Tyr
     1315                1320                1325

CTC AGC ATG GTA TAT AAC GGA GAT ATT CCA ACT ATC AAT TAC AAA GCC  4032
Leu Ser Met Val Tyr Asn Gly Asp Ile Pro Thr Ile Asn Tyr Lys Ala
1330                1335                1340

GCA TCA AGT GAT TTA AAA ATC TAT ATC TCA CCA AAA TTA AGA ATT ATT  4080
Ala Ser Ser Asp Leu Lys Ile Tyr Ile Ser Pro Lys Leu Arg Ile Ile
1345                1350                1355                1360

CAT AAT GGA TAT GAA GGA CAG AAG CGC AAT CAA TGC AAT CTG ATG AAT  4128
His Asn Gly Tyr Glu Gly Gln Lys Arg Asn Gln Cys Asn Leu Met Asn
               1365                1370                1375

AAA TAT GGC AAA CTA GGT GAT AAA TTT ATT GTT TAT ACT AGC TTG GGG  4176
Lys Tyr Gly Lys Leu Gly Asp Lys Phe Ile Val Tyr Thr Ser Leu Gly
          1380                1385                1390

GTC AAT CCA AAT AAC TCG TCA AAT AAG CTC ATG TTT TAC CCC GTC TAT  4224
Val Asn Pro Asn Asn Ser Ser Asn Lys Leu Met Phe Tyr Pro Val Tyr
     1395                1400                1405

CAA TAT AGC GGA AAC ACC AGT GGA CTC AAT CAA GGG AGA CTA CTA TTC  4272
Gln Tyr Ser Gly Asn Thr Ser Gly Leu Asn Gln Gly Arg Leu Leu Phe
1410                1415                1420

CAC CGT GAC ACC ACT TAT CCA TCT AAA GTA GAA GCT TGG ATT CCT GGA  4320
His Arg Asp Thr Thr Tyr Pro Ser Lys Val Glu Ala Trp Ile Pro Gly
1425                1430                1435                1440

GCA AAA CGT TCT CTA ACC AAC CAA AAT GCC GCC ATT GGT GAT GAT TAT  4368
Ala Lys Arg Ser Leu Thr Asn Gln Asn Ala Ala Ile Gly Asp Asp Tyr
               1445                1450                1455

GCT ACA GAC TCT CTG AAT AAA CCG GAT GAT CTT AAG CAA TAT ATC TTT  4416
Ala Thr Asp Ser Leu Asn Lys Pro Asp Asp Leu Lys Gln Tyr Ile Phe
          1460                1465                1470

ATG ACT GAC AGT AAA GGG ACT GCT ACT GAT GTC TCA GGC CCA GTA GAG  4464
Met Thr Asp Ser Lys Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu
     1475                1480                1485

ATT AAT ACT GCA ATT TCT CCA GCA AAA GTT CAG ATA ATA GTC AAA GCG  4512
Ile Asn Thr Ala Ile Ser Pro Ala Lys Val Gln Ile Ile Val Lys Ala
1490                1495                1500
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GGT GGC AAG GAG CAA ACT TTT ACC GCA GAT AAA GAT GTC TCC ATT CAG  4560
Gly Gly Lys Glu Gln Thr Phe Thr Ala Asp Lys Asp Val Ser Ile Gln
1505                1510                1515                1520

CCA TCA CCT AGC TTT GAT GAA ATG AAT TAT CAA TTT AAT GCC CTT GAA  4608
Pro Ser Pro Ser Phe Asp Glu Met Asn Tyr Gln Phe Asn Ala Leu Glu
                1525                1530                1535

ATA GAC GGT TCT GGT CTG AAT TTT ATT AAC AAC TCA GCC AGT ATT GAT  4656
Ile Asp Gly Ser Gly Leu Asn Phe Ile Asn Asn Ser Ala Ser Ile Asp
            1540                1545                1550

GTT ACT TTT ACC GCA TTT GCG GAG GAT GGC CGC AAA CTG GGT TAT GAA  4704
Val Thr Phe Thr Ala Phe Ala Glu Asp Gly Arg Lys Leu Gly Tyr Glu
        1555                1560                1565

AGT TTC AGT ATT CCT GTT ACC CTC AAG GTA AGT ACC GAT AAT GCC CTG  4752
Ser Phe Ser Ile Pro Val Thr Leu Lys Val Ser Thr Asp Asn Ala Leu
    1570                1575                1580

ACC CTG CAC CAT AAT GAA AAT GGT GCG CAA TAT ATG CAA TGG CAA TCC  4800
Thr Leu His His Asn Glu Asn Gly Ala Gln Tyr Met Gln Trp Gln Ser
1585                1590                1595                1600

TAT CGT ACC CGC CTG AAT ACT CTA TTT GCC CGC CAG TTG GTT GCA CGC  4848
Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Ala Arg
                1605                1610                1615

GCC ACC ACC GGA ATC GAT ACA ATT CTG AGT ATG GAA ACT CAG AAT ATT  4896
Ala Thr Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Asn Ile
            1620                1625                1630

CAG GAA CCG CAG TTA GGC AAA GGT TTC TAT GCT ACG TTC GTG ATA CCT  4944
Gln Glu Pro Gln Leu Gly Lys Gly Phe Tyr Ala Thr Phe Val Ile Pro
        1635                1640                1645

CCC TAT AAC CTA TCA ACT CAT GGT GAT GAA CGT TGG TTT AAG CTT TAT  4992
Pro Tyr Asn Leu Ser Thr His Gly Asp Glu Arg Trp Phe Lys Leu Tyr
    1650                1655                1660

ATC AAA CAT GTT GTT GAT AAT AAT TCA CAT ATT ATC TAT TCA GGC CAG  5040
Ile Lys His Val Val Asp Asn Asn Ser His Ile Ile Tyr Ser Gly Gln
1665                1670                1675                1680

CTA ACA GAT ACA AAT ATA AAC ATC ACA TTA TTT ATT CCT CTT GAT GAT  5088
Leu Thr Asp Thr Asn Ile Asn Ile Thr Leu Phe Ile Pro Leu Asp Asp
                1685                1690                1695

GTC CCA TTG AAT CAA GAT TAT CAC GCC AAG GTT TAT ATG ACC TTC AAG  5136
Val Pro Leu Asn Gln Asp Tyr His Ala Lys Val Tyr Met Thr Phe Lys
            1700                1705                1710

AAA TCA CCA TCA GAT GGT ACC TGG TGG GGC CCT CAC TTT GTT AGA GAT  5184
Lys Ser Pro Ser Asp Gly Thr Trp Trp Gly Pro His Phe Val Arg Asp
        1715                1720                1725

GAT AAA GGA ATA GTA ACA ATA AAC CCT AAA TCC ATT TTG ACC CAT TTT  5232
Asp Lys Gly Ile Val Thr Ile Asn Pro Lys Ser Ile Leu Thr His Phe
    1730                1735                1740

GAG AGC GTC AAT GTC CTG AAT AAT ATT AGT AGC GAA CCA ATG GAT TTC  5280
Glu Ser Val Asn Val Leu Asn Asn Ile Ser Ser Glu Pro Met Asp Phe
1745                1750                1755                1760

AGC GGC GCT AAC AGC CTC TAT TTC TGG GAA CTG TTC TAC TAT ACC CCG  5328
Ser Gly Ala Asn Ser Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro
                1765                1770                1775

ATG CTG GTT GCT CAA CGT TTG CTG CAT GAA CAG AAC TTC GAT GAA GCC  5376
Met Leu Val Ala Gln Arg Leu Leu His Glu Gln Asn Phe Asp Glu Ala
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                1730                1785                1790

AAC CGT TGG CTG AAA TAT GTC TGG AGT CCA TCC GGT TAT ATT GTC CAC  5424
Asn Arg Trp Leu Lys Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val His
        1795                1800                1805

GGC CAG ATT CAG AAC TAC CAG TGG AAC GTC CGC CCG TTA CTG GAA GAC  5472
Gly Gln Ile Gln Asn Tyr Gln Trp Asn Val Arg Pro Leu Leu Glu Asp
    1810                1815                1820

ACC AGT TGG AAC AGT GAT CCT TTG GAT TCC GTC GAT CCT GAC GCG GTA  5520
Thr Ser Trp Asn Ser Asp Pro Leu Asp Ser Val Asp Pro Asp Ala Val
1825                1830                1835                1840

GCA CAG CAC GAT CCA ATG CAC TAC AAA GTT TCA ACT TTT ATG CGT ACC  5568
Ala Gln His Asp Pro Met His Tyr Lys Val Ser Thr Phe Met Arg Thr
                1845                1850                1855

TTG GAT CTA TTG ATA GCA CGC GGC GAC CAT GCT TAT CGC CAA CTG GAA  5616
Leu Asp Leu Leu Ile Ala Arg Gly Asp His Ala Tyr Arg Gln Leu Glu
            1860                1865                1870

CGA GAT ACA CTC AAC GAA GCG AAG ATG TGG TAT ATG CAA GCG CTG CAT  5664
Arg Asp Thr Leu Asn Glu Ala Lys Met Trp Tyr Met Gln Ala Leu His
        1875                1880                1885

CTA TTA GGT GAC AAA CCT TAT CTA CCG CTG AGT ACG ACA TGG AGT GAT  5712
Leu Leu Gly Asp Lys Pro Tyr Leu Pro Leu Ser Thr Thr Trp Ser Asp
    1890                1895                1900

CCA CGA CTA GAC AGA GCC GCG GAT ATC ACT ACC CAA AAT GCT CAC GAC  5760
Pro Arg Leu Asp Arg Ala Ala Asp Ile Thr Thr Gln Asn Ala His Asp
1905                1910                1915                1920

AGC GCA ATA GTC GCT CTG CGG CAG AAT ATA CCT ACA CCG GCA CCT TTA  5808
Ser Ala Ile Val Ala Leu Arg Gln Asn Ile Pro Thr Pro Ala Pro Leu
                1925                1930                1935

TCA TTG CGC AGC GCT AAT ACC CTG ACT GAT CTC TTC CTG CCG CAA ATC  5856
Ser Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile
            1940                1945                1950

AAT GAA GTG ATG ATG AAT TAC TGG CAG ACA TTA GCT CAG AGA GTA TAC  5904
Asn Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr
        1955                1960                1965

AAT CTG CGT CAT AAC CTC TCT ATC GAC GGC CAG CCG TTA TAT CTG CCA  5952
Asn Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro
    1970                1975                1980

ATC TAT GCC ACA CCG GCC GAT CCG AAA GCG TTA CTC AGC GCC GCC GTT  6000
Ile Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val
1985                1990                1995                2000

GCC ACT TCT CAA GGT GGA GGC AAG CTA CCG GAA TCA TTT ATG TCC CTG  6048
Ala Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu
                2005                2010                2015

TGG CGT TTC CCG CAC ATG CTG GAA AAT GCG CGC GGC ATG GTT AGC CAG  6096
Trp Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln
            2020                2025                2030

CTC ACC CAG TTC GGC TCC ACG TTA CAA AAT ATT ATC GAA CGT CAG GAC  6144
Leu Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp
        2035                2040                2045

GCG GAA GCG CTC AAT GCG TTA TTA CAA AAT CAG GCC GCC GAG CTG ATA  6192
Ala Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile
    2050                2055                2060

TTG ACT AAC CTG AGC ATT CAG GAC AAA ACC ATT GAA GAA TTG GAT GCC  6240
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Leu Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala
2065                2070                2075                2080

GAG AAA ACG GTG TTG GAA AAA TCC AAA GCG GGA GCA CAA TCG CGC TTT  6288
Glu Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe
                    2085                2090                2095

GAT AGC TAC GGC AAA CTG TAC GAT GAG AAT ATC AAC GCC GGT GAA AAC  6336
Asp Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn
            2100                2105                2110

CAA GCC ATG ACG CTA CGA GCG TCC GCC GCC GGG CTT ACC ACG GCA GTT  6384
Gln Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val
        2115                2120                2125

CAG GCA TCC CGT CTG GCC GGT GCG GCG GCT GAT CTG GTG CCT AAC ATC  6432
Gln Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile
    2130                2135                2140

TTC GGC TTT GCC GGT GGC GGC AGC CGT TGG GGG GCT ATC GCT GAG GCG  6480
Phe Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala
2145                2150                2155                2160

ACA GGT TAT GTG ATG GAA TTC TCC GCG AAT GTT ATG AAC ACC GAA GCG  6528
Thr Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala
                    2165                2170                2175

GAT AAA ATT AGC CAA TCT GAA ACC TAC CGT CGT CGC CGT CAG GAG TGG  6576
Asp Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp
            2180                2185                2190

GAG ATC CAG CGG AAT AAT GCC GAA GCG GAA TTG AAG CAA ATC GAT GCT  6624
Glu Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala
        2195                2200                2205

CAG CTC AAA TCA CTC GCT GTA CGC CGC GAA GCC GCC GTA TTG CAG AAA  6672
Gln Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys
    2210                2215                2220

ACC AGT CTG AAA ACC CAA CAA GAA CAG ACC CAA TCT CAA TTG GCC TTC  6720
Thr Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe
2225                2230                2235                2240

CTG CAA CGT AAG TTC AGC AAT CAG GCG TTA TAC AAC TGG CTG CGT GGT  6768
Leu Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly
                    2245                2250                2255

CGA CTG GCG GCG ATT TAC TTC CAG TTC TAC GAT TTG GCC GTC GCG CGT  6816
Arg Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg
            2260                2265                2270

TGC CTG ATG GCA GAA CAA GCT TAC CGT TGG GAA CTC AAT GAT GAC TCT  6864
Cys Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser
        2275                2280                2285

GCC CGC TTC ATT AAA CCG GGC GCC TGG CAG GGA ACC TAT GCC GGT CTG  6912
Ala Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu
    2290                2295                2300

CTT GCA GGT GAA ACC TTG ATG CTG AGT CTG GCA CAA ATG GAA GAC GCT  6960
Leu Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala
2305                2310                2315                2320

CAT CTG AAA CGC GAT AAA CGC GCA TTA GAG GTT GAA CGC ACA GTA TCG  7008
His Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser
                    2325                2330                2335

CTG GCC GAA GTT TAT GCA GGA TTA CCA AAA GAT AAC GGT CCA TTT TCC  7056
Leu Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser
            2340                2345                2350
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CTG GCT CAG GAA ATT GAC AAG CTG GTG AGT CAA GGT TCA GGC AGT GCC  7104
Leu Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala
        2355                2360                2365

GGC AGT GGT AAT AAT AAT TTG GCG TTC GGC GCC GGC ACG GAC ACT AAA  7152
Gly Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys
    2370                2375                2380

ACC TCT TTG CAG GCA TCA GTT TCA TTC GCT GAT TTG AAA ATT CGT GAA  7200
Thr Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu
2385                2390                2395                2400

GAT TAC CCG GCA TCG CTT GGC AAA ATT CGA CGT ATC AAA CAG ATC AGC  7248
Asp Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser
                2405                2410                2415

GTC ACT TTG CCC GCG CTA CTG GGA CCG TAT CAG GAT GTA CAG GCA ATA  7296
Val Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile
            2420                2425                2430

TTG TCT TAC GGC GAT AAA GCC GGA TTA GCT AAC GGC TGT GAA GCG CTG  7344
Leu Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu
        2435                2440                2445

GCA GTT TCT CAC GGT ATG AAT GAC AGC GGC CAA TTC CAG CTC GAT TTC  7392
Ala Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe
    2450                2455                2460

AAC GAT GGC AAA TTC CTG CCA TTC GAA GGC ATC GCC ATT GAT CAA GGC  7440
Asn Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly
2465                2470                2475                2480

ACG CTG ACA CTG AGC TTC CCA AAT GCA TCT ATG CCG GAG AAA GGT AAA  7488
Thr Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys
                2485                2490                2495

CAA GCC ACT ATG TTA AAA ACC CTG AAC GAT ATC ATT TTG CAT ATT CGC  7536
Gln Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg
            2500                2505                2510

TAC ACC ATT AAA TAA      7551
Tyr Thr Ile Lys •••
            2516
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(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2516 amino acids
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47 (TcdA):

| Features | From | To | Description |
|---|---|---|---|
| Peptide | 1 | 2516 | TcdA proteins |
| Peptide | 89 | 1937 | $TcdA_{ii}$ peptide |
| Fragment | 89 | 100 | S2 N-terminus (SEQ ID NO:13) |
| Fragment | 284 | 299 | (SEQ ID NO:38) |
| Fragment | 554 | 563 | (SEQ ID NO:17) |
| Fragment | 1080 | 1092 | (SEQ ID NO:23; 12/13) |
| Fragment | 1385 | 1400 | (SEQ ID NO:18) |
| Fragment | 1478 | 1497 | (SEQ ID NO:39) |
| Fragment | 1620 | 1642 | (SEQ ID NO:21; 19/23) |
| Fragment | 1938 | 1948 | (SEQ ID NO:41) |
| Peptide | 1938 | 2516 | $TcdA_{iii}$ peptide |
| Fragment | 2327 | 2345 | (SEQ ID NO:42) |
| Fragment | 2398 | 2408 | (SEQ ID NO:43) |

Met Asn Glu Ser Val Lys Glu Ile Pro Asp Val Leu Lys Ser Gln Cys
1 5 10 15

Gly Phe Asn Cys Leu Thr Asp Ile Ser His Ser Ser Phe Asn Glu Phe
20 25 30

Arg Gln Gln Val Ser Glu His Leu Ser Trp Ser Glu Thr His Asp Leu
35 40 45

Tyr His Asp Ala Gln Gln Ala Gln Lys Asp Asn Arg Leu Tyr Glu Ala
50 55 60

Arg Ile Leu Lys Arg Ala Asn Pro Gln Leu Gln Asn Ala Val His Leu
65 70 75 80

Ala Ile Leu Ala Pro Asn Ala Glu Leu Ile Gly Tyr Asn Asn Gln Phe
85 90 95

Ser Gly Arg Ala Ser Gln Tyr Val Ala Pro Gly Thr Val Ser Ser Met
100 105 110

Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Arg Asn
115 120 125

Leu His Ala Ser Asp Ser Val Tyr Tyr Leu Asp Thr Arg Arg Pro Asp
130 135 140

Leu Lys Ser Met Ala Leu Ser Gln Gln Asn Met Asp Ile Glu Leu Ser
145 150 155 160

Thr Leu Ser Leu Ser Asn Glu Leu Leu Leu Glu Ser Ile Lys Thr Glu
165 170 175

Ser Lys Leu Glu Asn Tyr Thr Lys Val Met Glu Met Leu Ser Thr Phe
180 185 190

Arg Pro Ser Gly Ala Thr Pro Tyr His Asp Ala Tyr Glu Asn Val Arg
195 200 205

Glu Val Ile Gln Leu Gln Asp Pro Gly Leu Glu Gln Leu Asn Ala Ser
210 215 220

Pro Ala Ile Ala Gly Leu Met His Gln Ala Ser Leu Leu Gly Ile Asn
225 230 235 240

Ala Ser Ile Ser Pro Glu Leu Phe Asn Ile Leu Thr Glu Glu Ile Thr
245 250 255

Glu Gly Asn Ala Glu Glu Leu Tyr Lys Lys Asn Phe Gly Asn Ile Glu
260 265 270

Pro Ala Ser Leu Ala Met Pro Glu Tyr Leu Lys Arg Tyr Tyr Asn Leu
275 280 285

Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly Lys Ala Ser Asn Phe Gly
290 295 300

Gln Gln Glu Tyr Ser Asn Asn Gln Leu Ile Thr Pro Val Val Asn Ser
305 310 315 320

Ser Asp Gly Thr Val Lys Val Tyr Arg Ile Thr Arg Glu Tyr Thr Thr
325 330 335

Asn Ala Tyr Gln Met Asp Val Glu Leu Phe Pro Phe Gly Gly Glu Asn
340 345 350

Tyr Arg Leu Asp Tyr Lys Phe Lys Asn Phe Tyr Asn Ala Ser Tyr Leu
355 360 365

Ser Ile Lys Leu Asn Asp Lys Arg Glu Leu Val Arg Thr Glu Gly Ala

370 375 380

Pro Gln Val Asn Ile Glu Tyr Ser Ala Asn Ile Thr Leu Asn Thr Ala
385 390 395 400

Asp Ile Ser Gln Pro Phe Glu Ile Gly Leu Thr Arg Val Leu Pro Ser
405 410 415

Gly Ser Trp Ala Tyr Ala Ala Ala Lys Phe Thr Val Glu Glu Tyr Asn
420 425 430

Gln Tyr Ser Phe Leu Leu Lys Leu Asn Lys Ala Ile Arg Leu Ser Arg
435 440 445

Ala Thr Glu Leu Ser Pro Thr Ile Leu Glu Gly Ile Val Arg Ser Val
450 455 460

Asn Leu Gln Leu Asp Ile Asn Thr Asp Val Leu Gly Lys Val Phe Leu
465 470 475 480

Thr Lys Tyr Tyr Met Gln Arg Tyr Ala Ile His Ala Glu Thr Ala Leu
485 490 495

Ile Leu Cys Asn Ala Pro Ile Ser Gln Arg Ser Tyr Asp Asn Gln Pro
500 505 510

Ser Gln Phe Asp Arg Leu Phe Asn Thr Pro Leu Leu Asn Gly Gln Tyr
515 520 525

Phe Ser Thr Gly Asp Glu Glu Ile Asp Leu Asn Ser Gly Ser Thr Gly
530 535 540

Asp Trp Arg Lys Thr Ile Leu Lys Arg Ala Phe Asn Ile Asp Asp Val
545 550 555 560

Ser Leu Phe Arg Leu Leu Lys Ile Thr Asp His Asp Asn Lys Asp Gly
565 570 575

Lys Ile Lys Asn Asn Leu Lys Asn Leu Ser Asn Leu Tyr Ile Gly Lys
580 585 590

Leu Leu Ala Asp Ile His Gln Leu Thr Ile Asp Glu Leu Asp Leu Leu
595 600 605

Leu Ile Ala Val Gly Glu Gly Lys Thr Asn Leu Ser Ala Ile Ser Asp
610 615 620

Lys Gln Leu Ala Thr Leu Ile Arg Lys Leu Asn Thr Ile Thr Ser Trp
625 630 635 640

Leu His Thr Gln Lys Trp Ser Val Phe Gln Leu Phe Ile Met Thr Ser
645 650 655

Thr Ser Tyr Asn Lys Thr Leu Thr Pro Glu Ile Lys Asn Leu Leu Asp
660 665 670

Thr Val Tyr His Gly Leu Gln Gly Phe Asp Lys Asp Lys Ala Asp Leu
675 680 685

Leu His Val Met Ala Pro Tyr Ile Ala Ala Thr Leu Gln Leu Ser Ser
690 695 700

Glu Asn Val Ala His Ser Val Leu Leu Trp Ala Asp Lys Leu Gln Pro
705 710 715 720

Gly Asp Gly Ala Met Thr Ala Glu Lys Phe Trp Asp Trp Leu Asn Thr
725 730 735

Lys Tyr Thr Pro Gly Ser Ser Glu Ala Val Glu Thr Gln Glu His Ile
740 745 750

Val Gln Tyr Cys Gln Ala Leu Ala Gln Leu Glu Met Val Tyr His Ser
755 760 765

Thr Gly Ile Asn Glu Asn Ala Phe Arg Leu Phe Val Thr Lys Pro Glu
770 775 780

Met Phe Gly Ala Ala Thr Gly Ala Ala Pro Ala His Asp Ala Leu Ser
785 790 795 800

Leu Ile Met Leu Thr Arg Phe Ala Asp Trp Val Asn Ala Leu Gly Glu
805 810 815

Lys Ala Ser Ser Val Leu Ala Ala Phe Glu Ala Asn Ser Leu Thr Ala
820 825 830

Glu Gln Leu Ala Asp Ala Met Asn Leu Asp Ala Asn Leu Leu Leu Gln
835 840 845

Ala Ser Ile Gln Ala Gln Asn His Gln His Leu Pro Pro Val Thr Pro
850 855 860

Glu Asn Ala Phe Ser Cys Trp Thr Ser Ile Asn Thr Ile Leu Gln Trp
865 870 875 880

Val Asn Val Ala Gln Gln Leu Asn Val Ala Pro Gln Gly Val Ser Ala
885 890 895

Leu Val Gly Leu Asp Tyr Ile Gln Ser Met Lys Glu Thr Pro Thr Tyr
900 905 910

Ala Gln Trp Glu Asn Ala Ala Gly Val Leu Thr Ala Gly Leu Asn Ser
915 920 925

Gln Gln Ala Asn Thr Leu His Ala Phe Leu Asp Glu Ser Arg Ser Ala
930 935 940

Ala Leu Ser Thr Tyr Tyr Ile Arg Gln Val Ala Lys Ala Ala Ala Ala
945 950 955 960

Ile Lys Ser Arg Asp Asp Leu Tyr Gln Tyr Leu Leu Ile Asp Asn Gln
965 970 975

Val Ser Ala Ala Ile Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Ser
980 985 990

Ile Gln Leu Tyr Val Asn Arg Ala Leu Glu Asn Val Glu Glu Asn Ala
995 1000 1005

Asn Ser Gly Val Ile Ser Arg Gln Phe Phe Ile Asp Trp Asp Lys Tyr
1010 1015 1020

Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Gln Leu Val Tyr Tyr
1025 1030 1035 1040

Pro Glu Asn Tyr Ile Asp Pro Thr Met Arg Ile Gly Gln Thr Lys Met
1045 1050 1055

Met Asp Ala Leu Leu Gln Ser Val Ser Gln Ser Gln Leu Asn Ala Asp
1060 1065 1070

Thr Val Glu Asp Ala Phe Met Ser Tyr Leu Thr Ser Phe Glu Gln Val
1075 1080 1085

Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Ile Asn Asn Asp
1090 1095 1100

Gln Gly Leu Thr Tyr Phe Ile Gly Leu Ser Glu Thr Asp Ala Gly Glu
1105 1110 1115 1120

Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Phe Asn Asp Gly Lys Phe
1125 1130 1135

Ala Ala Asn Ala Trp Ser Glu Trp His Lys Ile Asp Cys Pro Ile Asn
1140 1145 1150

Pro Tyr Lys Ser Thr Ile Arg Pro Val Ile Tyr Lys Ser Arg Leu Tyr
1155 1160 1165

Leu Leu Trp Leu Glu Gln Lys Glu Ile Thr Lys Gln Thr Gly Asn Ser
1170 1175 1180

Lys Asp Gly Tyr Gln Thr Glu Thr Asp Tyr Arg Tyr Glu Leu Lys Leu
1185 1190 1195 1200

Ala His Ile Arg Tyr Asp Gly Thr Trp Asn Thr Pro Ile Thr Phe Asp
1205 1210 1215

Val Asn Lys Lys Ile Ser Glu Leu Lys Leu Glu Lys Asn Arg Ala Pro
1220 1225 1230

Gly Leu Tyr Cys Ala Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met
1235 1240 1245

Phe Tyr Asn Gln Gln Asp Thr Leu Asp Ser Tyr Lys Asn Ala Ser Met
1250 1255 1260

Gln Gly Leu Tyr Ile Phe Ala Asp Met Ala Ser Lys Asp Met Thr Pro
1265 1270 1275 1280

Glu Gln Ser Asn Val Tyr Arg Asp Asn Ser Tyr Gln Gln Phe Asp Thr
1285 1290 1295

Asn Asn Val Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr Glu Ile
1300 1305 1310

Pro Ser Ser Val Ser Ser Arg Lys Asp Tyr Gly Trp Gly Asp Tyr Tyr
1315 1320 1325

Leu Ser Met Val Tyr Asn Gly Asp Ile Pro Thr Ile Asn Tyr Lys Ala
1330 1335 1340

Ala Ser Ser Asp Leu Lys Ile Tyr Ile Ser Pro Lys Leu Arg Ile Ile
1345 1350 1355 1360

His Asn Gly Tyr Glu Gly Gln Lys Arg Asn Gln Cys Asn Leu Met Asn
1365 1370 1375

Lys Tyr Gly Lys Leu Gly Asp Lys Phe Ile Val Tyr Thr Ser Leu Gly
1380 1385 1390

Val Asn Pro Asn Asn Ser Ser Asn Lys Leu Met Phe Tyr Pro Val Tyr
1395 1400 1405

Gln Tyr Ser Gly Asn Thr Ser Gly Leu Asn Gln Gly Arg Leu Leu Phe
1410 1415 1420

His Arg Asp Thr Thr Tyr Pro Ser Lys Val Glu Ala Trp Ile Pro Gly
1425 1430 1435 1440

Ala Lys Arg Ser Leu Thr Asn Gln Asn Ala Ala Ile Gly Asp Asp Tyr
1445 1450 1455

Ala Thr Asp Ser Leu Asn Lys Pro Asp Asp Leu Lys Gln Tyr Ile Phe
1460 1465 1470

Met Thr Asp Ser Lys Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu
1475 1480 1485

Ile Asn Thr Ala Ile Ser Pro Ala Lys Val Gln Ile Ile Val Lys Ala
1490 1495 1500

Gly Gly Lys Glu Gln Thr Phe Thr Ala Asp Lys Asp Val Ser Ile Gln

1505 1510 1515 1520

Pro Ser Pro Ser Phe Asp Glu Met Asn Tyr Gln Phe Asn Ala Leu Glu
1525 1530 1535

Ile Asp Gly Ser Gly Leu Asn Phe Ile Asn Asn Ser Ala Ser Ile Asp
1540 1545 1550

Val Thr Phe Thr Ala Phe Ala Glu Asp Gly Arg Lys Leu Gly Tyr Glu
1555 1560 1565

Ser Phe Ser Ile Pro Val Thr Leu Lys Val Ser Thr Asp Asn Ala Leu
1570 1575 1580

Thr Leu His His Asn Glu Asn Gly Ala Gln Tyr Met Gln Trp Gln Ser
1585 1590 1595 1600

Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Ala Arg
1605 1610 1615

Ala Thr Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Asn Ile
1620 1625 1630

Gln Glu Pro Gln Leu Gly Lys Gly Phe Tyr Ala Thr Phe Val Ile Pro
1635 1640 1645

Pro Tyr Asn Leu Ser Thr His Gly Asp Glu Arg Trp Phe Lys Leu Tyr
1650 1655 1660

Ile Lys His Val Val Asp Asn Asn Ser His Ile Ile Tyr Ser Gly Gln
1665 1670 1675 1680

Leu Thr Asp Thr Asn Ile Asn Ile Thr Leu Phe Ile Pro Leu Asp Asp
1685 1690 1695

Val Pro Leu Asn Gln Asp Tyr His Ala Lys Val Tyr Met Thr Phe Lys
1700 1705 1710

Lys Ser Pro Ser Asp Gly Thr Trp Trp Gly Pro His Phe Val Arg Asp
1715 1720 1725

Asp Lys Gly Ile Val Thr Ile Asn Pro Lys Ser Ile Leu Thr His Phe
1730 1735 1740

Glu Ser Val Asn Val Leu Asn Asn Ile Ser Ser Glu Pro Met Asp Phe
1745 1750 1755 1760

Ser Gly Ala Asn Ser Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro
1765 1770 1775

Met Leu Val Ala Gln Arg Leu Leu His Glu Gln Asn Phe Asp Glu Ala
1780 1785 1790

Asn Arg Trp Leu Lys Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val His
1795 1800 1805

Gly Gln Ile Gln Asn Tyr Gln Trp Asn Val Arg Pro Leu Leu Glu Asp
1810 1815 1820

Thr Ser Trp Asn Ser Asp Pro Leu Asp Ser Val Asp Pro Asp Ala Val
1825 1830 1835 1840

Ala Gln His Asp Pro Met His Tyr Lys Val Ser Thr Phe Met Arg Thr
1845 1850 1855

Leu Asp Leu Leu Ile Ala Arg Gly Asp His Ala Tyr Arg Gln Leu Glu
1860 1865 1870

Arg Asp Thr Leu Asn Glu Ala Lys Met Trp Tyr Met Gln Ala Leu His
1875 1880 1885

Leu Leu Gly Asp [redacted] Pro Tyr Leu Pro Leu Ser Thr Th p Ser Asp
1890 1895 1900

Pro Arg Leu Asp Arg Ala Ala Asp Ile Thr Thr Gln Asn Ala His Asp
1905 1910 1915 1920

Ser Ala Ile Val Ala Leu Arg Gln Asn Ile Pro Thr Pro Ala Pro Leu
1925 1930 1935

Ser Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile
1940 1945 1950

Asn Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr
1955 1960 1965

Asn Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro
1970 1975 1980

Ile Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val
1985 1990 1995 2000

Ala Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu
2005 2010 2015

Trp Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln
2020 2025 2030

Leu Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp
2035 2040 2045

Ala Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile
2050 2055 2060

Leu Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala
2065 2070 2075 2080

Glu Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe
2085 2090 2095

Asp Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn
2100 2105 2110

Gln Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val
2115 2120 2125

Gln Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile
2130 2135 2140

Phe Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala
2145 2150 2155 2160

Thr Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala
2165 2170 2175

Asp Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp
2180 2185 2190

Glu Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala
2195 2200 2205

Gln Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys
2210 2215 2220

Thr Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe
2225 2230 2235 2240

Leu Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly
2245 2250 2255

Arg Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg
2260 2265 2270

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Cys Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser
        2275                2280                2285

Ala Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu
    2290                2295                2300

Leu Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala
2305                2310                2315                2320

His Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser
                2325                2330                2335

Leu Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser
            2340                2345                2350

Leu Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala
        2355                2360                2365

Gly Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys
    2370                2375                2380

Thr Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu
2385                2390                2395                2400

Asp Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser
                2405                2410                2415

Val Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile
            2420                2425                2430

Leu Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu
        2435                2440                2445

Ala Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe
    2450                2455                2460

Asn Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly
2465                2470                2475                2480

Thr Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys
                2485                2490                2495

Gln Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg
            2500                2505                2510

Tyr Thr Ile Lys
        2516
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(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5547 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48 ($tcdA_{ii}$ coding region):

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CTG ATA GGC TAT AAC AAT CAA TTT AGC GGT AGA GCC AGT CAA TAT GTT 48
Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Arg Ala Ser Gln Tyr Val
1               5                  10                  15

GCG CCG GGT ACC GTT TCT TCC ATG TTC TCC CCC GCC GCT TAT TTG ACT 96
Ala Pro Gly Thr Val Ser Ser Met Ph  Ser Pro Ala Ala Tyr Leu Thr
            20                  25                  30
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GAA CTT TAT CGT GAA GCA CGC AAT TTA CAC GCA AGT GAC TCC GTT TAT 144
Glu Leu Tyr Arg Glu Ala Arg Asn Leu His Ala Ser Asp Ser Val Tyr
        35                  40                  45

TAT CTG GAT ACC CGC CGC CCA GAT CTC AAA TCA ATG GCG CTC AGT CAG 192
Tyr Leu Asp Thr Arg Arg Pro Asp Leu Lys Ser Met Ala Leu Ser Gln
    50                  55                  60

CAA AAT ATG GAT ATA GAA TTA TCC ACA CTC TCT TTG TCC AAT GAG CTG 240
Gln Asn Met Asp Ile Glu Leu Ser Thr Leu Ser Leu Ser Asn Glu Leu
65                  70                  75                  80

TTA TTG GAA AGC ATT AAA ACT GAA TCT AAA CTG GAA AAC TAT ACT AAA 288
Leu Leu Glu Ser Ile Lys Thr Glu Ser Lys Leu Glu Asn Tyr Thr Lys
                85                  90                  95

GTG ATG GAA ATG CTC TCC ACT TTC CGT CCT TCC GGC GCA ACG CCT TAT 336
Val Met Glu Met Leu Ser Thr Phe Arg Pro Ser Gly Ala Thr Pro Tyr
            100                 105                 110

CAT GAT GCT TAT GAA AAT GTG CGT GAA GTT ATC CAG CTA CAA GAT CCT 384
His Asp Ala Tyr Glu Asn Val Arg Glu Val Ile Gln Leu Gln Asp Pro
        115                 120                 125

GGA CTT GAG CAA CTC AAT GCA TCA CCG GCA ATT GCC GGG TTG ATG CAT 432
Gly Leu Glu Gln Leu Asn Ala Ser Pro Ala Ile Ala Gly Leu Met His
    130                 135                 140

CAA GCC TCC CTA TTG GGT ATT AAC GCT TCA ATC TCG CCT GAG CTA TTT 480
Gln Ala Ser Leu Leu Gly Ile Asn Ala Ser Ile Ser Pro Glu Leu Phe
145                 150                 155                 160

AAT ATT CTG ACG GAG GAG ATT ACC GAA GGT AAT GCT GAG GAA CTT TAT 528
Asn Ile Leu Thr Glu Glu Ile Thr Glu Gly Asn Ala Glu Glu Leu Tyr
                165                 170                 175

AAG AAA AAT TTT GGT AAT ATC GAA CCG GCC TCA TTG GCT ATG CCG GAA 576
Lys Lys Asn Phe Gly Asn Ile Glu Pro Ala Ser Leu Ala Met Pro Glu
            180                 185                 190

TAC CTT AAA CGT TAT TAT AAT TTA AGC GAT GAA GAA CTT AGT CAG TTT 624
Tyr Leu Lys Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe
        195                 200                 205

ATT GGT AAA GCC AGC AAT TTT GGT CAA CAG GAA TAT AGT AAT AAC CAA 672
Ile Gly Lys Ala Ser Asn Phe Gly Gln Gln Glu Tyr Ser Asn Asn Gln
    210                 215                 220

CTT ATT ACT CCG GTA GTC AAC AGC AGT GAT GGC ACG GTT AAG GTA TAT 720
Leu Ile Thr Pro Val Val Asn Ser Ser Asp Gly Thr Val Lys Val Tyr
225                 230                 235                 240

CGG ATC ACC CGC GAA TAT ACA ACC AAT GCT TAT CAA ATG GAT GTG GAG 768
Arg Ile Thr Arg Glu Tyr Thr Thr Asn Ala Tyr Gln Met Asp Val Glu
                245                 250                 255

CTA TTT CCC TTC GGT GGT GAG AAT TAT CGG TTA GAT TAT AAA TTC AAA 816
Leu Phe Pro Phe Gly Gly Glu Asn Tyr Arg Leu Asp Tyr Lys Phe Lys
            260                 265                 270

AAT TTT TAT AAT GCC TCT TAT TTA TCC ATC AAG TTA AAT GAT AAA AGA 864
Asn Phe Tyr Asn Ala Ser Tyr Leu Ser Ile Lys Leu Asn Asp Lys Arg
        275                 280                 285

GAA CTT GTT CGA ACT GAA GGC GCT CCT CAA GTC AAT ATA GAA TAC TCC 912
Glu Leu Val Arg Thr Glu Gly Ala Pro Gln Val Asn Ile Glu Tyr Ser
    290                 295                 300

GCA AAT ATC ACA TTA AAT ACC GCT GAT ATC AGT CAA CCT TTT GAA ATT 960
Ala Asn Ile Thr Leu Asn Thr Ala Asp Ile Ser Gln Pro Phe Glu Ile
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      305                 310                 315                 320

      GGC CTG ACA CGA GTA CTT CCT TCC GGT TCT TGG GCA TAT GCC GCC GCA 1008
      Gly Leu Thr Arg Val Leu Pro Ser Gly Ser Trp Ala Tyr Ala Ala Ala
                      325                 330                 335

      AAA TTT ACC GTT GAA GAG TAT AAC CAA TAC TCT TTT CTG CTA AAA CTT 1056
      Lys Phe Thr Val Glu Glu Tyr Asn Gln Tyr Ser Phe Leu Leu Lys Leu
                  340                 345                 350

      AAC AAG GCT ATT CGT CTA TCA CGT GCG ACA GAA TTG TCA CCC ACG ATT 1104
      Asn Lys Ala Ile Arg Leu Ser Arg Ala Thr Glu Leu Ser Pro Thr Ile
              355                 360                 365

      CTG GAA GGC ATT GTG CGC AGT GTT AAT CTA CAA CTG GAT ATC AAC ACA 1152
      Leu Glu Gly Ile Val Arg Ser Val Asn Leu Gln Leu Asp Ile Asn Thr
          370                 375                 380

      GAC GTA TTA GGT AAA GTT TTT CTG ACT AAA TAT TAT ATG CAG CGT TAT 1200
      Asp Val Leu Gly Lys Val Phe Leu Thr Lys Tyr Tyr Met Gln Arg Tyr
      385                 390                 395                 400

      GCT ATT CAT GCT GAA ACT GCC CTG ATA CTA TGC AAC GCG CCT ATT TCA 1248
      Ala Ile His Ala Glu Thr Ala Leu Ile Leu Cys Asn Ala Pro Ile Ser
                      405                 410                 415

      CAA CGT TCA TAT GAT AAT CAA CCT AGC CAA TTT GAT CGC CTG TTT AAT 1296
      Gln Arg Ser Tyr Asp Asn Gln Pro Ser Gln Phe Asp Arg Leu Phe Asn
                  420                 425                 430

      ACG CCA TTA CTG AAC GGA CAA TAT TTT TCT ACC GGC GAT GAG GAG ATT 1344
      Thr Pro Leu Leu Asn Gly Gln Tyr Phe Ser Thr Gly Asp Glu Glu Ile
              435                 440                 445

      GAT TTA AAT TCA GGT AGC ACC GGC GAT TGG CGA AAA ACC ATA CTT AAG 1392
      Asp Leu Asn Ser Gly Ser Thr Gly Asp Trp Arg Lys Thr Ile Leu Lys
          450                 455                 460

      CGT GCA TTT AAT ATT GAT GAT GTC TCG CTC TTC CGC CTG CTT AAA ATT 1440
      Arg Ala Phe Asn Ile Asp Asp Val Ser Leu Phe Arg Leu Leu Lys Ile
      465                 470                 475                 480

      ACC GAC CAT GAT AAT AAA GAT GGA AAA ATT AAA AAT AAC CTA AAG AAT 1488
      Thr Asp His Asp Asn Lys Asp Gly Lys Ile Lys Asn Asn Leu Lys Asn
                      485                 490                 495

      CTT TCC AAT TTA TAT ATT GGA AAA TTA CTG GCA GAT ATT CAT CAA TTA 1536
      Leu Ser Asn Leu Tyr Ile Gly Lys Leu Leu Ala Asp Ile His Gln Leu
                  500                 505                 510

      ACC ATT GAT GAA CTG GAT TTA TTA CTG ATT GCC GTA GGT GAA GGA AAA 1584
      Thr Ile Asp Glu Leu Asp Leu Leu Leu Ile Ala Val Gly Glu Gly Lys
              515                 520                 525

      ACT AAT TTA TCC GCT ATC AGT GAT AAG CAA TTG GCT ACC CTG ATC AGA 1632
      Thr Asn Leu Ser Ala Ile Ser Asp Lys Gln Leu Ala Thr Leu Ile Arg
          530                 535                 540

      AAA CTC AAT ACT ATT ACC AGC TGG CTA CAT ACA CAG AAG TGG AGT GTA 1680
      Lys Leu Asn Thr Ile Thr Ser Trp Leu His Thr Gln Lys Trp Ser Val
      545                 550                 555                 560

      TTC CAG CTA TTT ATC ATG ACC TCC ACC AGC TAT AAC AAA ACG CTA ACG 1728
      Phe Gln Leu Phe Ile Met Thr Ser Thr Ser Tyr Asn Lys Thr Leu Thr
                      565                 570                 575

      CCT GAA ATT AAG AAT TTG CTG GAT ACC GTC TAC CAC GGT TTA CAA GGT 1776
      Pro Glu Ile Lys Asn Leu Leu Asp Thr Val Tyr His Gly Leu Gln Gly
                  580                 585                 590

      TTT GAT AAA GAC AAA GCA GAT TTG CTA CAT GTC ATG GCG CCC TAT ATT 1824
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Phe Asp Lys Asp Lys Ala Asp Leu Leu His Val Met Ala Pro Tyr Ile
595                                 600                 605

GCG GCC ACC TTG CAA TTA TCA TCG GAA AAT GTC GCC CAC TCG GTA CTC 1872
Ala Ala Thr Leu Gln Leu Ser Ser Glu Asn Val Ala His Ser Val Leu
610                 615                 620

CTT TGG GCA GAT AAG TTA CAG CCC GGC GAC GGC GCA ATG ACA GCA GAA 1920
Leu Trp Ala Asp Lys Leu Gln Pro Gly Asp Gly Ala Met Thr Ala Glu
625                 630                 635                 640

AAA TTC TGG GAC TGG TTG AAT ACT AAG TAT ACG CCG GGT TCA TCG GAA 1968
Lys Phe Trp Asp Trp Leu Asn Thr Lys Tyr Thr Pro Gly Ser Ser Glu
                645                 650                 655

GCC GTA GAA ACG CAG GAA CAT ATC GTT CAG TAT TGT CAG GCT CTG GCA 2016
Ala Val Glu Thr Gln Glu His Ile Val Gln Tyr Cys Gln Ala Leu Ala
            660                 665                 670

CAA TTG GAA ATG GTT TAC CAT TCC ACC GGC ATC AAC GAA AAC GCC TTC 2064
Gln Leu Glu Met Val Tyr His Ser Thr Gly Ile Asn Glu Asn Ala Phe
        675                 680                 685

CGT CTA TTT GTG ACA AAA CCA GAG ATG TTT GGC GCT GCA ACT GGA GCA 2112
Arg Leu Phe Val Thr Lys Pro Glu Met Phe Gly Ala Ala Thr Gly Ala
    690                 695                 700

GCG CCC GCG CAT GAT GCC CTT TCA CTG ATT ATG CTG ACA CGT TTT GCG 2160
Ala Pro Ala His Asp Ala Leu Ser Leu Ile Met Leu Thr Arg Phe Ala
705                 710                 715                 720

GAT TGG GTG AAC GCA CTA GGC GAA AAA GCG TCC TCG GTG CTA GCG GCA 2208
Asp Trp Val Asn Ala Leu Gly Glu Lys Ala Ser Ser Val Leu Ala Ala
                725                 730                 735

TTT GAA GCT AAC TCG TTA ACG GCA GAA CAA CTG GCT GAT GCC ATG AAT 2256
Phe Glu Ala Asn Ser Leu Thr Ala Glu Gln Leu Ala Asp Ala Met Asn
            740                 745                 750

CTT GAT GCT AAT TTG CTG TTG CAA GCC AGT ATT CAA GCA CAA AAT CAT 2304
Leu Asp Ala Asn Leu Leu Leu Gln Ala Ser Ile Gln Ala Gln Asn His
        755                 760                 765

CAA CAT CTT CCC CCA GTA ACT CCA GAA AAT GCG TTC TCC TGT TGG ACA 2352
Gln His Leu Pro Pro Val Thr Pro Glu Asn Ala Phe Ser Cys Trp Thr
    770                 775                 780

TCT ATC AAT ACT ATC CTG CAA TGG GTT AAT GTC GCA CAA CAA TTG AAT 2400
Ser Ile Asn Thr Ile Leu Gln Trp Val Asn Val Ala Gln Gln Leu Asn
785                 790                 795                 800

GTC GCC CCA CAG GGC GTT TCC GCT TTG GTC GGG CTG GAT TAT ATT CAA 2448
Val Ala Pro Gln Gly Val Ser Ala Leu Val Gly Leu Asp Tyr Ile Gln
                805                 810                 815

TCA ATG AAA GAG ACA CCG ACC TAT GCC CAG TGG GAA AAC GCG GCA GGC 2496
Ser Met Lys Glu Thr Pro Thr Tyr Ala Gln Trp Glu Asn Ala Ala Gly
            820                 825                 830

GTA TTA ACC GCC GGG TTG AAT TCA CAA CAG GCT AAT ACA TTA CAC GCT 2544
Val Leu Thr Ala Gly Leu Asn Ser Gln Gln Ala Asn Thr Leu His Ala
        835                 840                 845

TTT CTG GAT GAA TCT CGC AGT GCC GCA TTA AGC ACC TAC TAT ATC CGT 2592
Phe Leu Asp Glu Ser Arg Ser Ala Ala Leu Ser Thr Tyr Tyr Ile Arg
    850                 855                 860

CAA GTC GCC AAG GCA GCG GCG GCT ATT AAA AGC CGT GAT GAC TTG TAT 2640
Gln Val Ala Lys Ala Ala Ala Ala Ile Lys Ser Arg Asp Asp Leu Tyr
865                 870                 875                 880
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CAA TAC TTA CTG ATT GAT AAT CAG GTT TCT GCG GCA ATA AAA ACC ACC 2688
Gln Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Ala Ile Lys Thr Thr
            385                 390                 395

CGG ATC GCC GAA GCC ATT GCC AGT ATT CAA CTG TAC GTC AAC CGG GCA 2736
Arg Ile Ala Glu Ala Ile Ala Ser Ile Gln Leu Tyr Val Asn Arg Ala
        900                 905                 910

TTG GAA AAT GTG GAA GAA AAT GCC AAT TCG GGG GTT ATC AGC CGC CAA 2784
Leu Glu Asn Val Glu Glu Asn Ala Asn Ser Gly Val Ile Ser Arg Gln
    915                 920                 925

TTC TTT ATC GAC TGG GAC AAA TAC AAT AAA CGC TAC AGC ACT TGG GCG 2832
Phe Phe Ile Asp Trp Asp Lys Tyr Asn Lys Arg Tyr Ser Thr Trp Ala
930                 935                 940

GGT GTT TCT CAA TTA GTT TAC TAC CCG GAA AAC TAT ATT GAT CCG ACC 2880
Gly Val Ser Gln Leu Val Tyr Tyr Pro Glu Asn Tyr Ile Asp Pro Thr
945                 950                 955                 960

ATG CGT ATC GGA CAA ACC AAA ATG ATG GAC GCA TTA CTG CAA TCC GTC 2928
Met Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln Ser Val
            965                 970                 975

AGC CAA AGC CAA TTA AAC GCC GAT ACC GTC GAA GAT GCC TTT ATG TCT 2976
Ser Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe Met Ser
        980                 985                 990

TAT CTG ACA TCG TTT GAA CAA GTG GCT AAT CTT AAA GTT ATT AGC GCA 3024
Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile Ser Ala
    995                 1000                1005

TAT CAC GAT AAT ATT AAT AAC GAT CAA GGG CTG ACC TAT TTT ATC GGA 3072
Tyr His Asp Asn Ile Asn Asn Asp Gln Gly Leu Thr Tyr Phe Ile Gly
1010                1015                1020

CTC AGT GAA ACT GAT GCC GGT GAA TAT TAT TGG CGC AGT GTC GAT CAC 3120
Leu Ser Glu Thr Asp Ala Gly Glu Tyr Tyr Trp Arg Ser Val Asp His
1025                1030                1035                1040

AGT AAA TTC AAC GAC GGT AAA TTC GCG GCT AAT GCC TGG AGT GAA TGG 3168
Ser Lys Phe Asn Asp Gly Lys Phe Ala Ala Asn Ala Trp Ser Glu Trp
            1045                1050                1055

CAT AAA ATT GAT TGT CCA ATT AAC CCT TAT AAA AGC ACT ATC CGT CCA 3216
His Lys Ile Asp Cys Pro Ile Asn Pro Tyr Lys Ser Thr Ile Arg Pro
        1060                1065                1070

GTG ATA TAT AAA TCC CGC CTG TAT CTG CTC TGG TTG GAA CAA AAG GAG 3264
Val Ile Tyr Lys Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln Lys Glu
    1075                1080                1085

ATC ACC AAA CAG ACA GGA AAT AGT AAA GAT GGC TAT CAA ACT GAA ACG 3312
Ile Thr Lys Gln Thr Gly Asn Ser Lys Asp Gly Tyr Gln Thr Glu Thr
1090                1095                1100

GAT TAT CGT TAT GAA CTA AAA TTG GCG CAT ATC CGC TAT GAT GGC ACT 3360
Asp Tyr Arg Tyr Glu Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Thr
1105                1110                1115                1120

TGG AAT ACG CCA ATC ACC TTT GAT GTC AAT AAA AAA ATA TCC GAG CTA 3408
Trp Asn Thr Pro Ile Thr Phe Asp Val Asn Lys Lys Ile Ser Glu Leu
            1125                1130                1135

AAA CTG GAA AAA AAT AGA GCG CCC GGA CTC TAT TGT GCC GGT TAT CAA 3456
Lys Leu Glu Lys Asn Arg Ala Pro Gly Leu Tyr Cys Ala Gly Tyr Gln
        1140                1145                1150

GGT GAA GAT ACG TTG CTG GTG ATG TTT TAT AAC CAA CAA GAC ACA CTA 3504
Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Asn Gln Gln Asp Thr Leu
    1155                1160                1165
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GAT AGT TAT AAA AAC GCT TCA ATG CAA GGA CTA TAT ATC TTT GCT GAT 3552
Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp
    1170                1175                1180

ATG GCA TCC AAA GAT ATG ACC CCA GAA CAG AGC AAT GTT TAT CGG GAT 3600
Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp
1185                1190                1195                1200

AAT AGC TAT CAA CAA TTT GAT ACC AAT AAT GTC AGA AGA GTG AAT AAC 3648
Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn
                1205                1210                1215

CGC TAT GCA GAG GAT TAT GAG ATT CCT TCC TCG GTA AGT AGC CGT AAA 3696
Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys
            1220                1225                1230

GAC TAT GGT TGG GGA GAT TAT TAC CTC AGC ATG GTA TAT AAC GGA GAT 3744
Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp
        1235                1240                1245

ATT CCA ACT ATC AAT TAC AAA GCC GCA TCA AGT GAT TTA AAA ATC TAT 3792
Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr
    1250                1255                1260

ATC TCA CCA AAA TTA AGA ATT ATT CAT AAT GGA TAT GAA GGA CAG AAG 3840
Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys
1265                1270                1275                1280

CGC AAT CAA TGC AAT CTG ATG AAT AAA TAT GGC AAA CTA GGT GAT AAA 3888
Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys
                1285                1290                1295

TTT ATT GTT TAT ACT AGC TTG GGG GTC AAT CCA AAT AAC TCG TCA AAT 3936
Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn
            1300                1305                1310

AAG CTC ATG TTT TAC CCC GTC TAT CAA TAT AGC GGA AAC ACC AGT GGA 3984
Lys Leu Met Phe Tyr Pro Val Tyr Gln Tyr Ser Gly Asn Thr Ser Gly
        1315                1320                1325

CTC AAT CAA GGG AGA CTA CTA TTC CAC CGT GAC ACC ACT TAT CCA TCT 4032
Leu Asn Gln Gly Arg Leu Leu Phe His Arg Asp Thr Thr Tyr Pro Ser
    1330                1335                1340

AAA GTA GAA GCT TGG ATT CCT GGA GCA AAA CGT TCT CTA ACC AAC CAA 4080
Lys Val Glu Ala Trp Ile Pro Gly Ala Lys Arg Ser Leu Thr Asn Gln
1345                1350                1355                1360

AAT GCC GCC ATT GGT GAT GAT TAT GCT ACA GAC TCT CTG AAT AAA CCG 4128
Asn Ala Ala Ile Gly Asp Asp Tyr Ala Thr Asp Ser Leu Asn Lys Pro
                1365                1370                1375

GAT GAT CTT AAG CAA TAT ATC TTT ATG ACT GAC AGT AAA GGG ACT GCT 4176
Asp Asp Leu Lys Gln Tyr Ile Phe Met Thr Asp Ser Lys Gly Thr Ala
            1380                1385                1390

ACT GAT GTC TCA GGC CCA GTA GAG ATT AAT ACT GCA ATT TCT CCA GCA 4224
Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala Ile Ser Pro Ala
        1395                1400                1405

AAA GTT CAG ATA ATA GTC AAA GCG GGT GGC AAG GAG CAA ACT TTT ACC 4272
Lys Val Gln Ile Ile Val Lys Ala Gly Gly Lys Glu Gln Thr Phe Thr
    1410                1415                1420

GCA GAT AAA GAT GTC TCC ATT CAG CCA TCA CCT AGC TTT GAT GAA ATG 4320
Ala Asp Lys Asp Val Ser Ile Gln Pro Ser Pro Ser Phe Asp Glu Met
1425                1430                1435                1440

AAT TAT CAA TTT AAT GCC CTT GAA ATA GAC GGT TCT GGT CTG AAT TTT 4368
Asn Tyr Gln Phe Asn Ala Leu Glu Ile Asp Gly Ser Gly Leu Asn Phe
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                    1445                1450                1455

ATT AAC AAC TCA GCC AGT ATT GAT GTT ACT TTT ACC GCA TTT GCG GAG 4416
Ile Asn Asn Ser Ala Ser Ile Asp Val Thr Phe Thr Ala Phe Ala Glu
            1460                1465                1470

GAT GGC CGC AAA CTG GGT TAT GAA AGT TTC AGT ATT CCT GTT ACC CTC 4464
Asp Gly Arg Lys Leu Gly Tyr Glu Ser Phe Ser Ile Pro Val Thr Leu
        1475                1480                1485

AAG GTA AGT ACC GAT AAT GCC CTG ACC CTG CAC CAT AAT GAA AAT GGT 4512
Lys Val Ser Thr Asp Asn Ala Leu Thr Leu His His Asn Glu Asn Gly
    1490                1495                1500

GCG CAA TAT ATG CAA TGG CAA TCC TAT CGT ACC CGC CTG AAT ACT CTA 4560
Ala Gln Tyr Met Gln Trp Gln Ser Tyr Arg Thr Arg Leu Asn Thr Leu
1505                1510                1515                1520

TTT GCC CGC CAG TTG GTT GCA CGC GCC ACC ACC GGA ATC GAT ACA ATT 4608
Phe Ala Arg Gln Leu Val Ala Arg Ala Thr Thr Gly Ile Asp Thr Ile
                1525                1530                1535

CTG AGT ATG GAA ACT CAG AAT ATT CAG GAA CCG CAG TTA GGC AAA GGT 4656
Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly Lys Gly
            1540                1545                1550

TTC TAT GCT ACG TTC GTG ATA CCT CCC TAT AAC CTA TCA ACT CAT GGT 4704
Phe Tyr Ala Thr Phe Val Ile Pro Pro Tyr Asn Leu Ser Thr His Gly
        1555                1560                1565

GAT GAA CGT TGG TTT AAG CTT TAT ATC AAA CAT GTT GTT GAT AAT AAT 4752
Asp Glu Arg Trp Phe Lys Leu Tyr Ile Lys His Val Val Asp Asn Asn
    1570                1575                1580

TCA CAT ATT ATC TAT TCA GGC CAG CTA ACA GAT ACA AAT ATA AAC ATC 4800
Ser His Ile Ile Tyr Ser Gly Gln Leu Thr Asp Thr Asn Ile Asn Ile
1585                1590                1595                1600

ACA TTA TTT ATT CCT CTT GAT GAT GTC CCA TTG AAT CAA GAT TAT CAC 4848
Thr Leu Phe Ile Pro Leu Asp Asp Val Pro Leu Asn Gln Asp Tyr His
                1605                1610                1615

GCC AAG GTT TAT ATG ACC TTC AAG AAA TCA CCA TCA GAT GGT ACC TGG 4896
Ala Lys Val Tyr Met Thr Phe Lys Lys Ser Pro Ser Asp Gly Thr Trp
            1620                1625                1630

TGG GGC CCT CAC TTT GTT AGA GAT GAT AAA GGA ATA GTA ACA ATA AAC 4944
Trp Gly Pro His Phe Val Arg Asp Asp Lys Gly Ile Val Thr Ile Asn
        1635                1640                1645

CCT AAA TCC ATT TTG ACC CAT TTT GAG AGC GTC AAT GTC CTG AAT AAT 4992
Pro Lys Ser Ile Leu Thr His Phe Glu Ser Val Asn Val Leu Asn Asn
    1650                1655                1660

ATT AGT AGC GAA CCA ATG GAT TTC AGC GGC GCT AAC AGC CTC TAT TTC 5040
Ile Ser Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ser Leu Tyr Phe
1665                1670                1675                1680

TGG GAA CTG TTC TAC TAT ACC CCG ATG CTG GTT GCT CAA CGT TTG CTG 5088
Trp Glu Leu Phe Tyr Tyr Thr Pro Met Leu Val Ala Gln Arg Leu Leu
                1685                1690                1695

CAT GAA CAG AAC TTC GAT GAA GCC AAC CGT TGG CTG AAA TAT GTC TGG 5136
His Glu Gln Asn Phe Asp Glu Ala Asn Arg Trp Leu Lys Tyr Val Trp
            1700                1705                1710

AGT CCA TCC GGT TAT ATT GTC CAC GGC CAG ATT CAG AAC TAC CAG TGG 5184
Ser Pro Ser Gly Tyr Ile Val His Gly Gln Ile Gln Asn Tyr Gln Trp
        1715                1720                1725

AAC GTC CGC CCG TTA CTG GAA GAC ACC AGT TGG AAC AGT GAT CCT TTG 5232
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```
Asn Val Arg Pro Leu Leu Glu Asp Thr Ser Trp Asn Ser Asp Pro Leu
    1730                1735                1740

GAT TCC GTC GAT CCT GAC GCG GTA GCA CAG CAC GAT CCA ATG CAC TAC 5230
Asp Ser Val Asp Pro Asp Ala Val Ala Gln His Asp Pro Met His Tyr
1745                1750                1755                1760

AAA GTT TCA ACT TTT ATG CGT ACC TTG GAT CTA TTG ATA GCA CGC GGC 5328
Lys Val Ser Thr Phe Met Arg Thr Leu Asp Leu Leu Ile Ala Arg Gly
                1765                1770                1775

GAC CAT GCT TAT CGC CAA CTG GAA CGA GAT ACA CTC AAC GAA GCG AAG 5376
Asp His Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Asn Glu Ala Lys
            1780                1785                1790

ATG TGG TAT ATG CAA GCG CTG CAT CTA TTA GGT GAC AAA CCT TAT CTA 5424
Met Trp Tyr Met Gln Ala Leu His Leu Leu Gly Asp Lys Pro Tyr Leu
        1795                1800                1805

CCG CTG AGT ACG ACA TGG AGT GAT CCA CGA CTA GAC AGA GCC GCG GAT 5472
Pro Leu Ser Thr Thr Trp Ser Asp Pro Arg Leu Asp Arg Ala Ala Asp
    1810                1815                1820

ATC ACT ACC CAA AAT GCT CAC GAC AGC GCA ATA GTC GCT CTG CGG CAG 5520
Ile Thr Thr Gln Asn Ala His Asp Ser Ala Ile Val Ala Leu Arg Gln
1825                1830                1835                1840

AAT ATA CCT ACA CCG GCA CCT TTA TCA   5547
Asn Ile Pro Thr Pro Ala Pro Leu Ser
                1845                1849
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1849 amino acids
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49 ($TcdA_{ii}$):

| Features | From | To | Description |
|---|---|---|---|
| Peptide | 1 | 1849 | $TcdA_{ii}$ peptide |
| Fragment | 1 | 12 | S2 N-terminus (SEQ ID NO:13) |
| Fragment | 196 | 211 | (SEQ ID NO:38) |
| Fragment | 466 | 475 | (SEQ ID NO:17) |
| Fragment | 993 | 1004 | (SEQ ID NO:23; 12/13) |
| Fragment | 1297 | 1312 | (SEQ ID NO:18) |
| Fragment | 1390 | 1409 | (SEQ ID NO:39) |
| Fragment | 1532 | 1554 | (SEQ ID NO:21; 19/23) |

```
Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Arg Ala Ser Gln Tyr Val
1               5                  10                  15

Ala Pro Gly Thr Val Ser Ser Met Phe Ser Pro Ala Ala Tyr Leu Thr
            20                  25                  30

Glu Leu Tyr Arg Glu Ala Arg Asn Leu His Ala Ser Asp Ser Val Tyr
        35                  40                  45

Tyr Leu Asp Thr Arg Arg Pro Asp Leu Lys Ser Met Ala Leu Ser Gln
    50                  55                  60

Gln Asn Met Asp Ile Glu Leu Ser Thr Leu Ser Leu Ser Asn Glu Leu
65                  70                  75                  80

Leu Leu Glu Ser Ile Lys Thr Glu Ser Lys Leu Glu Asn Tyr Thr Lys
```

85 90 95

Val Met Glu Met Leu Ser Thr Phe Arg Pro Ser Gly Ala Thr Pro Tyr
100 105 110

His Asp Ala Tyr Glu Asn Val Arg Glu Val Ile Gln Leu Gln Asp Pro
115 120 125

Gly Leu Glu Gln Leu Asn Ala Ser Pro Ala Ile Ala Gly Leu Met His
130 135 140

Gln Ala Ser Leu Leu Gly Ile Asn Ala Ser Ile Ser Pro Glu Leu Phe
145 150 155 160

Asn Ile Leu Thr Glu Glu Ile Thr Glu Gly Asn Ala Glu Glu Leu Tyr
165 170 175

Lys Lys Asn Phe Gly Asn Ile Glu Pro Ala Ser Leu Ala Met Pro Glu
180 185 190

Tyr Leu Lys Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe
195 200 205

Ile Gly Lys Ala Ser Asn Phe Gly Gln Gln Glu Tyr Ser Asn Asn Gln
210 215 220

Leu Ile Thr Pro Val Val Asn Ser Ser Asp Gly Thr Val Lys Val Tyr
225 230 235 240

Arg Ile Thr Arg Glu Tyr Thr Thr Asn Ala Tyr Gln Met Asp Val Glu
245 250 255

Leu Phe Pro Phe Gly Gly Glu Asn Tyr Arg Leu Asp Tyr Lys Phe Lys
260 265 270

Asn Phe Tyr Asn Ala Ser Tyr Leu Ser Ile Lys Leu Asn Asp Lys Arg
275 280 285

Glu Leu Val Arg Thr Glu Gly Ala Pro Gln Val Asn Ile Glu Tyr Ser
290 295 300

Ala Asn Ile Thr Leu Asn Thr Ala Asp Ile Ser Gln Pro Phe Glu Ile
305 310 315 320

Gly Leu Thr Arg Val Leu Pro Ser Gly Ser Trp Ala Tyr Ala Ala Ala
325 330 335

Lys Phe Thr Val Glu Glu Tyr Asn Gln Tyr Ser Phe Leu Leu Lys Leu
340 345 350

Asn Lys Ala Ile Arg Leu Ser Arg Ala Thr Glu Leu Ser Pro Thr Ile
355 360 365

Leu Glu Gly Ile Val Arg Ser Val Asn Leu Gln Leu Asp Ile Asn Thr
370 375 380

Asp Val Leu Gly Lys Val Phe Leu Thr Lys Tyr Tyr Met Gln Arg Tyr
385 390 395 400

Ala Ile His Ala Glu Thr Ala Leu Ile Leu Cys Asn Ala Pro Ile Ser
405 410 415

Gln Arg Ser Tyr Asp Asn Gln Pro Ser Gln Phe Asp Arg Leu Phe Asn
420 425 430

Thr Pro Leu Leu Asn Gly Gln Tyr Phe Ser Thr Gly Asp Glu Glu Ile
435 440 445

Asp Leu Asn Ser Gly Ser Thr Gly Asp Trp Arg Lys Thr Ile Leu Lys
450 455 460

Arg Ala Phe As[redacted]e Asp Asp Val Ser Leu Phe Arg Leu Lys Ile
465 470 475 480

Thr Asp His Asp Asn Lys Asp Gly Lys Ile Lys Asn Asn Leu Lys Asn
485 490 495

Leu Ser Asn Leu Tyr Ile Gly Lys Leu Leu Ala Asp Ile His Gln Leu
500 505 510

Thr Ile Asp Glu Leu Asp Leu Leu Leu Ile Ala Val Gly Glu Gly Lys
515 520 525

Thr Asn Leu Ser Ala Ile Ser Asp Lys Gln Leu Ala Thr Leu Ile Arg
530 535 540

Lys Leu Asn Thr Ile Thr Ser Trp Leu His Thr Gln Lys Trp Ser Val
545 550 555 560

Phe Gln Leu Phe Ile Met Thr Ser Thr Ser Tyr Asn Lys Thr Leu Thr
565 570 575

Pro Glu Ile Lys Asn Leu Leu Asp Thr Val Tyr His Gly Leu Gln Gly
580 585 590

Phe Asp Lys Asp Lys Ala Asp Leu Leu His Val Met Ala Pro Tyr Ile
595 600 605

Ala Ala Thr Leu Gln Leu Ser Ser Glu Asn Val Ala His Ser Val Leu
610 615 620

Leu Trp Ala Asp Lys Leu Gln Pro Gly Asp Gly Ala Met Thr Ala Glu
625 630 635 640

Lys Phe Trp Asp Trp Leu Asn Thr Lys Tyr Thr Pro Gly Ser Ser Glu
645 650 655

Ala Val Glu Thr Gln Glu His Ile Val Gln Tyr Cys Gln Ala Leu Ala
660 665 670

Gln Leu Glu Met Val Tyr His Ser Thr Gly Ile Asn Glu Asn Ala Phe
675 680 685

Arg Leu Phe Val Thr Lys Pro Glu Met Phe Gly Ala Ala Thr Gly Ala
690 695 700

Ala Pro Ala His Asp Ala Leu Ser Leu Ile Met Leu Thr Arg Phe Ala
705 710 715 720
Asp Trp Val Asn Ala Leu Gly Glu Lys Ala Ser Ser Val Leu Ala Ala
725 730 735

Phe Glu Ala Asn Ser Leu Thr Ala Glu Gln Leu Ala Asp Ala Met Asn
740 745 750

Leu Asp Ala Asn Leu Leu Leu Gln Ala Ser Ile Gln Ala Gln Asn His
755 760 765

Gln His Leu Pro Pro Val Thr Pro Glu Asn Ala Phe Ser Cys Trp Thr
770 775 780

Ser Ile Asn Thr Ile Leu Gln Trp Val Asn Val Ala Gln Gln Leu Asn
785 790 795 800

Val Ala Pro Gln Gly Val Ser Ala Leu Val Gly Leu Asp Tyr Ile Gln
805 810 815

Ser Met Lys Glu Thr Pro Thr Tyr Ala Gln Trp Glu Asn Ala Ala Gly
820 825 830

Val Leu Thr Ala Gly Leu Asn Ser Gln Gln Ala Asn Thr Leu His Ala
835 840 845

Phe Leu A lu Ser Arg Ser Ala Ala Leu Ser Tyr Tyr Ile Arg
850 855

Gln Val Ala Lys Ala Ala Ala Ala Ile Lys Ser Arg Asp Asp Leu Tyr
865 870 875 880

Gln Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Ala Ile Lys Thr Thr
885 890 895

Arg Ile Ala Glu Ala Ile Ala Ser Ile Gln Leu Tyr Val Asn Arg Ala
900 905 910

Leu Glu Asn Val Glu Glu Asn Ala Asn Ser Gly Val Ile Ser Arg Gln
915 920 925

Phe Phe Ile Asp Trp Asp Lys Tyr Asn Lys Arg Tyr Ser Thr Trp Ala
930 935 940

Gly Val Ser Gln Leu Val Tyr Tyr Pro Glu Asn Tyr Ile Asp Pro Thr
945 950 955 960

Met Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln Ser Val
965 970 975

Ser Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe Met Ser
980 985 990

Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile Ser Ala
995 1000 1005

Tyr His Asp Asn Ile Asn Asn Asp Gln Gly Leu Thr Tyr Phe Ile Gly
1010 1015 1020

Leu Ser Glu Thr Asp Ala Gly Glu Tyr Tyr Trp Arg Ser Val Asp His
1025 1030 1035 1040

Ser Lys Phe Asn Asp Gly Lys Phe Ala Ala Asn Ala Trp Ser Glu Trp
1045 1050 1055

His Lys Ile Asp Cys Pro Ile Asn Pro Tyr Lys Ser Thr Ile Arg Pro
1060 1065 1070

Val Ile Tyr Lys Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln Lys Glu
1075 1080 1085

Ile Thr Lys Gln Thr Gly Asn Ser Lys Asp Gly Tyr Gln Thr Glu Thr
1090 1095 1100

Asp Tyr Arg Tyr Glu Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Thr
1105 1110 1115 1120

Trp Asn Thr Pro Ile Thr Phe Asp Val Asn Lys Lys Ile Ser Glu Leu
1125 1130 1135

Lys Leu Glu Lys Asn Arg Ala Pro Gly Leu Tyr Cys Ala Gly Tyr Gln
1140 1145 1150

Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Asn Gln Gln Asp Thr Leu
1155 1160 1165

Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp
1170 1175 1180

Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp
1185 1190 1195 1200

Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn
1205 1210 1215

Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys
1220 1225 1230

Asp Tyr Gly T[redacted]ly Asp Tyr Tyr Leu Ser Met Val[redacted] Asn Gly Asp
1235 1240 1245

Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr
1250 1255 1260

Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys
1265 1270 1275 1280

Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys
1285 1290 1295

Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn
1300 1305 1310

Lys Leu Met Phe Tyr Pro Val Tyr Gln Tyr Ser Gly Asn Thr Ser Gly
1315 1320 1325

Leu Asn Gln Gly Arg Leu Leu Phe His Arg Asp Thr Thr Tyr Pro Ser
1330 1335 1340

Lys Val Glu Ala Trp Ile Pro Gly Ala Lys Arg Ser Leu Thr Asn Gln
1345 1350 1355 1360

Asn Ala Ala Ile Gly Asp Asp Tyr Ala Thr Asp Ser Leu Asn Lys Pro
1365 1370 1375

Asp Asp Leu Lys Gln Tyr Ile Phe Met Thr Asp Ser Lys Gly Thr Ala
1380 1385 1390

Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala Ile Ser Pro Ala
1395 1400 1405

Lys Val Gln Ile Ile Val Lys Ala Gly Gly Lys Glu Gln Thr Phe Thr
1410 1415 1420

Ala Asp Lys Asp Val Ser Ile Gln Pro Ser Pro Ser Phe Asp Glu Met
1425 1430 1435 1440

Asn Tyr Gln Phe Asn Ala Leu Glu Ile Asp Gly Ser Gly Leu Asn Phe
1445 1450 1455

Ile Asn Asn Ser Ala Ser Ile Asp Val Thr Phe Thr Ala Phe Ala Glu
1460 1465 1470

Asp Gly Arg Lys Leu Gly Tyr Glu Ser Phe Ser Ile Pro Val Thr Leu
1475 1480 1485

Lys Val Ser Thr Asp Asn Ala Leu Thr Leu His His Asn Glu Asn Gly
1490 1495 1500

Ala Gln Tyr Met Gln Trp Gln Ser Tyr Arg Thr Arg Leu Asn Thr Leu
1505 1510 1515 1520

Phe Ala Arg Gln Leu Val Ala Arg Ala Thr Thr Gly Ile Asp Thr Ile
1525 1530 1535

Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly Lys Gly
1540 1545 1550

Phe Tyr Ala Thr Phe Val Ile Pro Pro Tyr Asn Leu Ser Thr His Gly
1555 1560 1565

Asp Glu Arg Trp Phe Lys Leu Tyr Ile Lys His Val Val Asp Asn Asn
1570 1575 1580

Ser His Ile Ile Tyr Ser Gly Gln Leu Thr Asp Thr Asn Ile Asn Ile
1585 1590 1595 1600

Thr Leu Phe Ile Pro Leu Asp Asp Val Pro Leu Asn Gln Asp Tyr His

1605 1610 1615

Ala Lys Val Tyr Met Thr Phe Lys Lys Ser Pro Ser Asp Gly Thr Trp
1620 1625 1630

Trp Gly Pro His Phe Val Arg Asp Asp Lys Gly Ile Val Thr Ile Asn
1635 1640 1645

Pro Lys Ser Ile Leu Thr His Phe Glu Ser Val Asn Val Leu Asn Asn
1650 1655 1660

Ile Ser Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ser Leu Tyr Phe
1665 1670 1675 1680

Trp Glu Leu Phe Tyr Tyr Thr Pro Met Leu Val Ala Gln Arg Leu Leu
1685 1690 1695

His Glu Gln Asn Phe Asp Glu Ala Asn Arg Trp Leu Lys Tyr Val Trp
1700 1705 1710

Ser Pro Ser Gly Tyr Ile Val His Gly Gln Ile Gln Asn Tyr Gln Trp
1715 1720 1725

Asn Val Arg Pro Leu Leu Glu Asp Thr Ser Trp Asn Ser Asp Pro Leu
1730 1735 1740

Asp Ser Val Asp Pro Asp Ala Val Ala Gln His Asp Pro Met His Tyr
1745 1750 1755 1760

Lys Val Ser Thr Phe Met Arg Thr Leu Asp Leu Leu Ile Ala Arg Gly
1765 1770 1775

Asp His Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Asn Glu Ala Lys
1780 1785 1790

Met Trp Tyr Met Gln Ala Leu His Leu Leu Gly Asp Lys Pro Tyr Leu
1795 1800 1805

Pro Leu Ser Thr Thr Trp Ser Asp Pro Arg Leu Asp Arg Ala Ala Asp
1810 1815 1820

Ile Thr Thr Gln Asn Ala His Asp Ser Ala Ile Val Ala Leu Arg Gln
1825 1830 1835 1840

Asn Ile Pro Thr Pro Ala Pro Leu Ser
1845 1849

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1740 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50 (TcdAiii coding region):

TTG CGC AGC GCT AAT ACC CTG ACT GAT CTC TTC CTG CCG CAA ATC AAT 48
Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile Asn
1 5 10 15

GAA GTG ATG ATG AAT TAC TGG CAG ACA TTA GCT CAG AGA GTA TAC AAT 96
Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr Asn
20 25 30

CTG CGT CAT AAC CTC TCT ATC GAC GGC CAG CCG TTA TAT CTG CCA ATC 144

```
Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro Ile
          35                  40                  45

TAT GCC ACA CCG GCC GAT CCG AAA GCG TTA CTC AGC GCC GCC GTT GCC 192
Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ala
    50                  55                  60

ACT TCT CAA GGT GGA GGC AAG CTA CCG GAA TCA TTT ATG TCC CTG TGG 240
Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu Trp
65                  70                  75                  80

CGT TTC CCG CAC ATG CTG GAA AAT GCG CGC GGC ATG GTT AGC CAG CTC 288
Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln Leu
                85                  90                  95

ACC CAG TTC GGC TCC ACG TTA CAA AAT ATT ATC GAA CGT CAG GAC GCG 336
Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp Ala
            100                 105                 110

GAA GCG CTC AAT GCG TTA TTA CAA AAT CAG GCC GCC GAG CTG ATA TTG 384
Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile Leu
        115                 120                 125

ACT AAC CTG AGC ATT CAG GAC AAA ACC ATT GAA GAA TTG GAT GCC GAG 432
Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala Glu
    130                 135                 140

AAA ACG GTG TTG GAA AAA TCC AAA GCG GGA GCA CAA TCG CGC TTT GAT 480
Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe Asp
145                 150                 155                 160

AGC TAC GGC AAA CTG TAC GAT GAG AAT ATC AAC GCC GGT GAA AAC CAA 528
Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn Gln
                165                 170                 175

GCC ATG ACG CTA CGA GCG TCC GCC GCC GGG CTT ACC ACG GCA GTT CAG 576
Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val Gln
            180                 185                 190

GCA TCC CGT CTG GCC GGT GCG GCG GCT GAT CTG GTG CCT AAC ATC TTC 624
Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile Phe
        195                 200                 205

GGC TTT GCC GGT GGC GGC AGC CGT TGG GGG GCT ATC GCT GAG GCG ACA 672
Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala Thr
    210                 215                 220

GGT TAT GTG ATG GAA TTC TCC GCG AAT GTT ATG AAC ACC GAA GCG GAT 720
Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala Asp
225                 230                 235                 240

AAA ATT AGC CAA TCT GAA ACC TAC CGT CGT CGC CGT CAG GAG TGG GAG 768
Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp Glu
                245                 250                 255

ATC CAG CGG AAT AAT GCC GAA GCG GAA TTG AAG CAA ATC GAT GCT CAG 816
Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala Gln
            260                 265                 270

CTC AAA TCA CTC GCT GTA CGC CGC GAA GCC GCC GTA TTG CAG AAA ACC 864
Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys Thr
        275                 280                 285

AGT CTG AAA ACC CAA CAA GAA CAG ACC CAA TCT CAA TTG GCC TTC CTG 912
Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe Leu
    290                 295                 300

CAA CGT AAG TTC AGC AAT CAG GCG TTA TAC AAC TGG CTG CGT GGT CGA 960
Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly Arg
305                 310                 315                 320
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CTG GCG GCG ATT TAC TTC CAG TTC TAC GAT TTG GCC GTC GCG CGT TGC 1008
Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg Cys
            325                 330                 335

CTG ATG GCA GAA CAA GCT TAC CGT TGG GAA CTC AAT GAT GAC TCT GCC 1056
Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser Ala
        340                 345                 350

CGC TTC ATT AAA CCG GGC GCC TGG CAG GGA ACC TAT GCC GGT CTG CTT 1104
Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
    355                 360                 365

GCA GGT GAA ACC TTG ATG CTG AGT CTG GCA CAA ATG GAA GAC GCT CAT 1152
Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala His
370                 375                 380

CTG AAA CGC GAT AAA CGC GCA TTA GAG GTT GAA CGC ACA GTA TCG CTG 1200
Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
385                 390                 395                 400

GCC GAA GTT TAT GCA GGA TTA CCA AAA GAT AAC GGT CCA TTT TCC CTG 1248
Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser Leu
            405                 410                 415

GCT CAG GAA ATT GAC AAG CTG GTG AGT CAA GGT TCA GGC AGT GCC GGC 1296
Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala Gly
        420                 425                 430

AGT GGT AAT AAT AAT TTG GCG TTC GGC GCC GGC ACG GAC ACT AAA ACC 1344
Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys Thr
    435                 440                 445

TCT TTG CAG GCA TCA GTT TCA TTC GCT GAT TTG AAA ATT CGT GAA GAT 1392
Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu Asp
450                 455                 460

TAC CCG GCA TCG CTT GGC AAA ATT CGA CGT ATC AAA CAG ATC AGC GTC 1440
Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser Val
465                 470                 475                 480

ACT TTG CCC GCG CTA CTG GGA CCG TAT CAG GAT GTA CAG GCA ATA TTG 1488
Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile Leu
            485                 490                 495

TCT TAC GGC GAT AAA GCC GGA TTA GCT AAC GGC TGT GAA GCG CTG GCA 1536
Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu Ala
        500                 505                 510

GTT TCT CAC GGT ATG AAT GAC AGC GGC CAA TTC CAG CTC GAT TTC AAC 1584
Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn
    515                 520                 525

GAT GGC AAA TTC CTG CCA TTC GAA GGC ATC GCC ATT GAT CAA GGC ACG 1632
Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly Thr
530                 535                 540

CTG ACA CTG AGC TTC CCA AAT GCA TCT ATG CCG GAG AAA GGT AAA CAA 1680
Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys Gln
545                 550                 555                 560

GCC ACT ATG TTA AAA ACC CTG AAC GAT ATC ATT TTG CAT ATT CGC TAC 1728
Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg Tyr
            565                 570                 575

ACC ATT AAA TAA   1740
Thr Ile Lys ...
        579
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 579 amino acids
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51 (TcdA$_{iii}$):

```
Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile Asn
 1               5                  10                  15

Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr Asn
            20                  25                  30

Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro Ile
        35                  40                  45

Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ala
    50                  55                  60

Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu Trp
65                  70                  75                  80

Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln Leu
                85                  90                  95

Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp Ala
            100                 105                 110

Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile Leu
        115                 120                 125

Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala Glu
    130                 135                 140

Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe Asp
145                 150                 155                 160

Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn Gln
                165                 170                 175

Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val Gln
            180                 185                 190

Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile Phe
        195                 200                 205

Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala Thr
    210                 215                 220

Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala Asp
225                 230                 235                 240

Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp Glu
                245                 250                 255

Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala Gln
            260                 265                 270

Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys Thr
        275                 280                 285

Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe Leu
    290                 295                 300

Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly Arg
305                 310                 315                 320
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```
Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg Cys
                325                 330                 335

Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser Ala
            340                 345                 350

Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
        355                 360                 365

Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala His
    370                 375                 380

Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
385                 390                 395                 400

Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser Leu
                405                 410                 415

Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala Gly
            420                 425                 430

Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys Thr
        435                 440                 445

Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu Asp
    450                 455                 460

Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser Val
465                 470                 475                 480

Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile Leu
                485                 490                 495

Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu Ala
            500                 505                 510

Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn
        515                 520                 525

Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly Thr
    530                 535                 540

Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys Gln
545                 550                 555                 560

Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg Tyr
                565                 570                 575

Thr Ile Lys ...
        579
```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5532 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52 (TcdAiii coding region):

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TTT ATA CAA GGT TAT AGT GAT CTG TTT GGT AAT CGT GCT GAT AAC TAT 48
Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala Asp Asn Tyr
1                 5                  10                  15

GCC GCG CCG GGC TCG GTT GCA TCG ATG TTC TCA CCG GCG GCT TAT TTG 96
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     Ala Ala Pro Gly      Val Ala Ser Met Phe Ser Pro A    la Tyr Leu
                 20                  25                  30

     ACG GAA TTG TAC CGT GAA GCC AAA AAC TTG CAT GAC AGC AGC TCA ATT 144
     Thr Glu Leu Tyr Arg Glu Ala Lys Asn Leu His Asp Ser Ser Ser Ile
                 35                  40                  45

     TAT TAC CTA GAT AAA CGT CGC CCG GAT TTA GCA AGC TTA ATG CTC AGC 192
     Tyr Tyr Leu Asp Lys Arg Arg Pro Asp Leu Ala Ser Leu Met Leu Ser
         50                  55                  60

     CAG AAA AAT ATG GAT GAG GAA ATT TCA ACG CTG GCT CTC TCT AAT GAA 240
     Gln Lys Asn Met Asp Glu Glu Ile Ser Thr Leu Ala Leu Ser Asn Glu
     65                  70                  75                  80

     TTG TGC CTT GCC GGG ATC GAA ACA AAA ACA GGA AAA TCA CAA GAT GAA 288
     Leu Cys Leu Ala Gly Ile Glu Thr Lys Thr Gly Lys Ser Gln Asp Glu
                     85                  90                  95

     GTG ATG GAT ATG TTG TCA ACT TAT CGT TTA AGT GGA GAG ACA CCT TAT 336
     Val Met Asp Met Leu Ser Thr Tyr Arg Leu Ser Gly Glu Thr Pro Tyr
                 100                 105                 110

     CAT CAC GCT TAT GAA ACT GTT CGT GAA ATC GTT CAT GAA CGT GAT CCA 384
     His His Ala Tyr Glu Thr Val Arg Glu Ile Val His Glu Arg Asp Pro
             115                 120                 125

     GGA TTT CGT CAT TTG TCA CAG GCA CCC ATT GTT GCT GCT AAG CTC GAT 432
     Gly Phe Arg His Leu Ser Gln Ala Pro Ile Val Ala Ala Lys Leu Asp
         130                 135                 140

     CCT GTG ACT TTG TTG GGT ATT AGC TCC CAT ATT TCG CCA GAA CTG TAT 480
     Pro Val Thr Leu Leu Gly Ile Ser Ser His Ile Ser Pro Glu Leu Tyr
     145                 150                 155                 160

     AAC TTG CTG ATT GAG GAG ATC CCG GAA AAA GAT GAA GCC GCG CTT GAT 528
     Asn Leu Leu Ile Glu Glu Ile Pro Glu Lys Asp Glu Ala Ala Leu Asp
                     165                 170                 175

     ACG CTT TAT AAA ACA AAC TTT GGC GAT ATT ACT ACT GCT CAG TTA ATG 576
     Thr Leu Tyr Lys Thr Asn Phe Gly Asp Ile Thr Thr Ala Gln Leu Met
                 180                 185                 190

     TCC CCA AGT TAT CTG GCC CGG TAT TAT GGC GTC TCA CCG GAA GAT ATT 624
     Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr Gly Val Ser Pro Glu Asp Ile
             195                 200                 205

     GCC TAC GTG ACG ACT TCA TTA TCA CAT GTT GGA TAT AGC AGT GAT ATT 672
     Ala Tyr Val Thr Thr Ser Leu Ser His Val Gly Tyr Ser Ser Asp Ile
         210                 215                 220

     CTG GTT ATT CCG TTG GTC GAT GGT GTG GGT AAG ATG GAA GTA GTT CGT 720
     Leu Val Ile Pro Leu Val Asp Gly Val Gly Lys Met Glu Val Val Arg
     225                 230                 235                 240

     GTT ACC CGA ACA CCA TCG GAT AAT TAT ACC AGT CAG ACG AAT TAT ATT 768
     Val Thr Arg Thr Pro Ser Asp Asn Tyr Thr Ser Gln Thr Asn Tyr Ile
                     245                 250                 255

     GAG CTG TAT CCA CAG GGT GGC GAC AAT TAT TTG ATC AAA TAC AAT CTA 816
     Glu Leu Tyr Pro Gln Gly Gly Asp Asn Tyr Leu Ile Lys Tyr Asn Leu
                 260                 265                 270

     AGC AAT AGT TTT GGT TTG GAT GAT TTT TAT CTG CAA TAT AAA GAT GGT 864
     Ser Asn Ser Phe Gly Leu Asp Asp Phe Tyr Leu Gln Tyr Lys Asp Gly
             275                 280                 285

     TCC GCT GAT TGG ACT GAG ATT GCC CAT AAT CCC TAT CCT GAT ATG GTC 912
     Ser Ala Asp Trp Thr Glu Ile Ala His Asn Pro Tyr Pro Asp Met Val
         290                 295                 300
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ATA AAT CAA AAG TAT GAA TCA CAG GCG ACA ATC AAA AGT AGT GAC TCT 960
Ile Asn Gln Lys Tyr Glu Ser Gln Ala Thr Ile Lys Arg Ser Asp Ser
305                 310                 315                 320

GAC AAT ATA CTC AGT ATA GGG TTA CAA AGA TGG CAT AGC GGT AGT TAT 1008
Asp Asn Ile Leu Ser Ile Gly Leu Gln Arg Trp His Ser Gly Ser Tyr
                325                 330                 335

AAT TTT GCC GCC GCC AAT TTT AAA ATT GAC CAA TAC TCC CCG AAA GCT 1056
Asn Phe Ala Ala Ala Asn Phe Lys Ile Asp Gln Tyr Ser Pro Lys Ala
            340                 345                 350

TTC CTG CTT AAA ATG AAT AAG GCT ATT CGG TTG CTC AAA GCT ACC GGC 1104
Phe Leu Leu Lys Met Asn Lys Ala Ile Arg Leu Leu Lys Ala Thr Gly
        355                 360                 365

CTC TCT TTT GCT ACG TTG GAG CGT ATT GTT GAT AGT GTT AAT AGC ACC 1152
Leu Ser Phe Ala Thr Leu Glu Arg Ile Val Asp Ser Val Asn Ser Thr
    370                 375                 380

AAA TCC ATC ACG GTT GAG GTA TTA AAC AAG GTT TAT CGG GTA AAA TTC 1200
Lys Ser Ile Thr Val Glu Val Leu Asn Lys Val Tyr Arg Val Lys Phe
385                 390                 395                 400

TAT ATT GAT CGT TAT GGC ATC AGT GAA GAG ACA GCC GCT ATT TTG GCT 1248
Tyr Ile Asp Arg Tyr Gly Ile Ser Glu Glu Thr Ala Ala Ile Leu Ala
                405                 410                 415

AAT ATT AAT ATC TCT CAG CAA GCT GTT GGC AAT CAG CTT AGC CAG TTT 1296
Asn Ile Asn Ile Ser Gln Gln Ala Val Gly Asn Gln Leu Ser Gln Phe
            420                 425                 430

GAG CAA CTA TTT AAT CAC CCG CCG CTC AAT GGT ATT CGC TAT GAA ATC 1344
Glu Gln Leu Phe Asn His Pro Pro Leu Asn Gly Ile Arg Tyr Glu Ile
        435                 440                 445

AGT GAG GAC AAC TCC AAA CAT CTT CCT AAT CCT GAT CTG AAC CTT AAA 1392
Ser Glu Asp Asn Ser Lys His Leu Pro Asn Pro Asp Leu Asn Leu Lys
    450                 455                 460

CCA GAC AGT ACC GGT GAT GAT CAA CGC AAG GCG GTT TTA AAA CGC GCG 1440
Pro Asp Ser Thr Gly Asp Asp Gln Arg Lys Ala Val Leu Lys Arg Ala
465                 470                 475                 480

TTT CAG GTT AAC GCC AGT GAG TTG TAT CAG ATG TTA TTG ATC ACT GAT 1488
Phe Gln Val Asn Ala Ser Glu Leu Tyr Gln Met Leu Leu Ile Thr Asp
                485                 490                 495

CGT AAA GAA GAC GGT GTT ATC AAA AAT AAC TTA GAG AAT TTG TCT GAT 1536
Arg Lys Glu Asp Gly Val Ile Lys Asn Asn Leu Glu Asn Leu Ser Asp
            500                 505                 510

CTG TAT TTG GTT AGT TTG CTG GCC CAG ATT CAT AAC CTG ACT ATT GCT 1584
Leu Tyr Leu Val Ser Leu Leu Ala Gln Ile His Asn Leu Thr Ile Ala
        515                 520                 525

GAA TTG AAC ATT TTG TTG GTG ATT TGT GGC TAT GGC GAC ACC AAC ATT 1632
Glu Leu Asn Ile Leu Leu Val Ile Cys Gly Tyr Gly Asp Thr Asn Ile
    530                 535                 540

TAT CAG ATT ACC GAC GAT AAT TTA GCC AAA ATA GTG GAA ACA TTG TTG 1680
Tyr Gln Ile Thr Asp Asp Asn Leu Ala Lys Ile Val Glu Thr Leu Leu
545                 550                 555                 560

TGG ATC ACT CAA TGG TTG AAG ACC CAA AAA TGG ACA GTT ACC GAC CTG 1728
Trp Ile Thr Gln Trp Leu Lys Thr Gln Lys Trp Thr Val Thr Asp Leu
                565                 570                 575

TTT CTG ATG ACC ACG GCC ACT TAC AGC ACC ACT TTA ACG CCA GAA ATT 1776
Phe Leu Met Thr Thr Ala Thr Tyr Ser Thr Thr Leu Thr Pro Glu Ile
            580                 585                 590
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AGC AAT CTG ACG GCT ACG TTG TCT TCA ACT TTG CAT GGC AAA GAG AGT 1824
Ser Asn Leu Thr Ala Thr Leu Ser Ser Thr Leu His Gly Lys Glu Ser
        595                 600                 605

CTG ATT GGG GAA GAT CTG AAA AGA GCA ATG GCG CCT TGC TTC ACT TCG 1872
Leu Ile Gly Glu Asp Leu Lys Arg Ala Met Ala Pro Cys Phe Thr Ser
    610                 615                 620

GCT TTG CAT TTG ACT TCT CAA GAA GTT GCG TAT GAC CTG CTG TTG TGG 1920
Ala Leu His Leu Thr Ser Gln Glu Val Ala Tyr Asp Leu Leu Leu Trp
625                 630                 635                 640

ATA GAC CAG ATT CAA CCG GCA CAA ATA ACT GTT GAT GGG TTT TGG GAA 1968
Ile Asp Gln Ile Gln Pro Ala Gln Ile Thr Val Asp Gly Phe Trp Glu
                645                 650                 655

GAA GTG CAA ACA ACA CCA ACC AGC TTG AAG GTG ATT ACC TTT GCT CAG 2016
Glu Val Gln Thr Thr Pro Thr Ser Leu Lys Val Ile Thr Phe Ala Gln
            660                 665                 670

GTG CTG GCA CAA TTG AGC CTG ATC TAT CGT CGT ATT GGG TTA AGT GAA 2064
Val Leu Ala Gln Leu Ser Leu Ile Tyr Arg Arg Ile Gly Leu Ser Glu
        675                 680                 685

ACG GAA CTG TCA CTG ATC GTG ACT CAA TCT TCT CTG CTA GTG GCA GGC 2112
Thr Glu Leu Ser Leu Ile Val Thr Gln Ser Ser Leu Leu Val Ala Gly
    690                 695                 700

AAA AGC ATA CTG GAT CAC GGT CTG TTA ACC CTG ATG GCC TTG GAA GGT 2160
Lys Ser Ile Leu Asp His Gly Leu Leu Thr Leu Met Ala Leu Glu Gly
705                 710                 715                 720

TTT CAT ACC TGG GTT AAT GGC TTG GGG CAA CAT GCC TCC TTG ATA TTG 2208
Phe His Thr Trp Val Asn Gly Leu Gly Gln His Ala Ser Leu Ile Leu
                725                 730                 735

GCG GCG TTG AAA GAC GGA GCC TTG ACA GTT ACC GAT GTA GCA CAA GCT 2256
Ala Ala Leu Lys Asp Gly Ala Leu Thr Val Thr Asp Val Ala Gln Ala
            740                 745                 750

ATG AAT AAG GAG GAA TCT CTC CTA CAA ATG GCA GCT AAT CAG GTG GAG 2304
Met Asn Lys Glu Glu Ser Leu Leu Gln Met Ala Ala Asn Gln Val Glu
        755                 760                 765

AAG GAT CTA ACA AAA CTG ACC AGT TGG ACA CAG ATT GAC GCT ATT CTG 2352
Lys Asp Leu Thr Lys Leu Thr Ser Trp Thr Gln Ile Asp Ala Ile Leu
    770                 775                 780

CAA TGG TTA CAG ATG TCT TCG GCC TTG GCG GTT TCT CCA CTG GAT CTG 2400
Gln Trp Leu Gln Met Ser Ser Ala Leu Ala Val Ser Pro Leu Asp Leu
785                 790                 795                 800

GCA GGG ATG ATG GCC CTG AAA TAT GGG ATA GAT CAT AAC TAT GCT GCC 2448
Ala Gly Met Met Ala Leu Lys Tyr Gly Ile Asp His Asn Tyr Ala Ala
                805                 810                 815

TGG CAA GCT GCG GCG GCT GCG CTG ATG GCT GAT CAT GCT AAT CAG GCA 2496
Trp Gln Ala Ala Ala Ala Ala Leu Met Ala Asp His Ala Asn Gln Ala
            820                 825                 830

CAG AAA AAA CTG GAT GAG ACG TTC AGT AAG GCA TTA TGT AAC TAT TAT 2544
Gln Lys Lys Leu Asp Glu Thr Phe Ser Lys Ala Leu Cys Asn Tyr Tyr
        835                 840                 845

ATT AAT GCT GTT GTC GAT AGT GCT GCT GGA GTA CGT GAT CGT AAC GGT 2592
Ile Asn Ala Val Val Asp Ser Ala Ala Gly Val Arg Asp Arg Asn Gly
    350                 855                 860

TTA TAT ACC TAT TTG CTG ATT GAT AAT CAG GTT TCT GCC GAT GTG ATC 2640
Leu Tyr Thr Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Asp Val Ile
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      865                 870                 875                 880

      ACT TCA CGT ATT GCA GAA GCT ATC GCC GGT ATT CAA CTG TAC GTT AAC 2688
      Thr Ser Arg Ile Ala Glu Ala Ile Ala Gly Ile Gln Leu Tyr Val Asn
                      885                 890                 895

      CGG GCT TTA AAC CGA GAT GAA GGT CAG CTT GCA TCG GAC GTT AGT ACC 2736
      Arg Ala Leu Asn Arg Asp Glu Gly Gln Leu Ala Ser Asp Val Ser Thr
                  900                 905                 910

      CGT CAG TTC TTC ACT GAC TGG GAA CGT TAC AAT AAA CGT TAC AGT ACT 2784
      Arg Gln Phe Phe Thr Asp Trp Glu Arg Tyr Asn Lys Arg Tyr Ser Thr
              915                 920                 925

      TGG GCT GGT GTC TCT GAA CTG GTC TAT TAT CCA GAA AAC TAT GTT GAT 2832
      Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro Glu Asn Tyr Val Asp
          930                 935                 940

      CCC ACT CAG CGC ATT GGG CAA ACC AAA ATG ATG GAT GCG CTG TTG CAA 2880
      Pro Thr Gln Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln
      945                 950                 955                 960

      TCC ATC AAC CAG AGC CAG CTA AAT GCG GAT ACG GTG GAA GAT GCT TTC 2928
      Ser Ile Asn Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe
                      965                 970                 975

      AAA ACT TAT TTG ACC AGC TTT GAG CAG GTA GCA AAT CTG AAA GTA ATT 2976
      Lys Thr Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile
                  980                 985                 990

      AGT GCT TAC CAC GAT AAT GTG AAT GTG GAT CAA GGA TTA ACT TAT TTT 3024
      Ser Ala Tyr His Asp Asn Val Asn Val Asp Gln Gly Leu Thr Tyr Phe
              995                 1000                1005

      ATC GGT ATC GAC CAA GCA GCT CCG GGT ACG TAT TAC TGG CGT AGT GTT 3072
      Ile Gly Ile Asp Gln Ala Ala Pro Gly Thr Tyr Tyr Trp Arg Ser Val
          1010                1015                1020

      GAT CAC AGC AAA TGT GAA AAT GGC AAG TTT GCC GCT AAT GCT TGG GGT 3120
      Asp His Ser Lys Cys Glu Asn Gly Lys Phe Ala Ala Asn Ala Trp Gly
      1025                1030                1035                1040

      GAG TGG AAT AAA ATT ACC TGT GCT GTC AAT CCT TGG AAA AAT ATC ATC 3168
      Glu Trp Asn Lys Ile Thr Cys Ala Val Asn Pro Trp Lys Asn Ile Ile
                      1045                1050                1055

      CGT CCG GTT GTT TAT ATG TCC CGC TTA TAT CTG CTA TGG CTG GAG CAG 3216
      Arg Pro Val Val Tyr Met Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln
                  1060                1065                1070

      CAA TCA AAG AAA AGT GAT GAT GGT AAA ACC ACG ATT TAT CAA TAT AAC 3264
      Gln Ser Lys Lys Ser Asp Asp Gly Lys Thr Thr Ile Tyr Gln Tyr Asn
              1075                1080                1085

      TTA AAA CTG GCT CAT ATT CGT TAC GAC GGT AGT TGG AAT ACA CCA TTT 3312
      Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Ser Trp Asn Thr Pro Phe
          1090                1095                1100

      ACT TTT GAT GTG ACA GAA AAG GTA AAA AAT TAC ACG TCG AGT ACT GAT 3360
      Thr Phe Asp Val Thr Glu Lys Val Lys Asn Tyr Thr Ser Ser Thr Asp
      1105                1110                1115                1120

      GCT GCT GAA TCT TTA GGG TTG TAT TGT ACT GGT TAT CAA GGG GAA GAC 3408
      Ala Ala Glu Ser Leu Gly Leu Tyr Cys Thr Gly Tyr Gln Gly Glu Asp
                      1125                1130                1135

      ACT CTA TTA GTT ATG TTC TAT TCG ATG CAG AGT AGT TAT AGC TCC TAT 3456
      Thr Leu Leu Val Met Phe Tyr Ser Met Gln Ser Ser Tyr Ser Ser Tyr
                  1140                1145                1150

      ACC GAT AAT AAT GCG CCG GTC ACT GGG CTA TAT ATT TTC GCT GAT ATG 3504
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Thr Asp Asn Asn Ala Pro Val Thr Gly Leu Tyr Ile Phe Ala Asp Met
        1155                1160                1165

TCA TCA GAC AAT ATG ACG AAT GCA CAA GCA ACT AAC TAT TGG AAT AAC 3552
Ser Ser Asp Asn Met Thr Asn Ala Gln Ala Thr Asn Tyr Trp Asn Asn
    1170                1175                1180

AGT TAT CCG CAA TTT GAT ACT GTG ATG GCA GAT CCG GAT AGC GAC AAT 3600
Ser Tyr Pro Gln Phe Asp Thr Val Met Ala Asp Pro Asp Ser Asp Asn
1185                1190                1195                1200

AAA AAA GTC ATA ACC AGA AGA GTT AAT AAC CGT TAT GCG GAG GAT TAT 3648
Lys Lys Val Ile Thr Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr
                1205                1210                1215

GAA ATT CCT TCC TCT GTG ACA AGT AAC AGT AAT TAT TCT TGG GGT GAT 3696
Glu Ile Pro Ser Ser Val Thr Ser Asn Ser Asn Tyr Ser Trp Gly Asp
            1220                1225                1230

CAC AGT TTA ACC ATG CTT TAT GGT GGT AGT GTT CCT AAT ATT ACT TTT 3744
His Ser Leu Thr Met Leu Tyr Gly Gly Ser Val Pro Asn Ile Thr Phe
        1235                1240                1245

GAA TCG GCG GCA GAA GAT TTA AGG CTA TCT ACC AAT ATG GCA TTG AGT 3792
Glu Ser Ala Ala Glu Asp Leu Arg Leu Ser Thr Asn Met Ala Leu Ser
    1250                1255                1260

ATT ATT CAT AAT GGA TAT GCG GGA ACC CGC CGT ATA CAA TGT AAT CTT 3840
Ile Ile His Asn Gly Tyr Ala Gly Thr Arg Arg Ile Gln Cys Asn Leu
1265                1270                1275                1280

ATG AAA CAA TAC GCT TCA TTA GGT GAT AAA TTT ATA ATT TAT GAT TCA 3888
Met Lys Gln Tyr Ala Ser Leu Gly Asp Lys Phe Ile Ile Tyr Asp Ser
                1285                1290                1295

TCA TTT GAT GAT GCA AAC CGT TTT AAT CTG GTG CCA TTG TTT AAA TTC 3936
Ser Phe Asp Asp Ala Asn Arg Phe Asn Leu Val Pro Leu Phe Lys Phe
            1300                1305                1310

GGA AAA GAC GAG AAC TCA GAT GAT AGT ATT TGT ATA TAT AAT GAA AAC 3984
Gly Lys Asp Glu Asn Ser Asp Asp Ser Ile Cys Ile Tyr Asn Glu Asn
        1315                1320                1325

CCT TCC TCT GAA GAT AAG AAG TGG TAT TTT TCT TCG AAA GAT GAC AAT 4032
Pro Ser Ser Glu Asp Lys Lys Trp Tyr Phe Ser Ser Lys Asp Asp Asn
    1330                1335                1340

AAA ACA GCG GAT TAT AAT GGT GGA ACT CAA TGT ATA GAT GCT GGA ACC 4080
Lys Thr Ala Asp Tyr Asn Gly Gly Thr Gln Cys Ile Asp Ala Gly Thr
1345                1350                1355                1360

AGT AAC AAA GAT TTT TAT TAT AAT CTC CAG GAG ATT GAA GTA ATT AGT 4128
Ser Asn Lys Asp Phe Tyr Tyr Asn Leu Gln Glu Ile Glu Val Ile Ser
                1365                1370                1375

GTT ACT GGT GGG TAT TGG TCG AGT TAT AAA ATA TCC AAC CCG ATT AAT 4176
Val Thr Gly Gly Tyr Trp Ser Ser Tyr Lys Ile Ser Asn Pro Ile Asn
            1380                1385                1390

ATC AAT ACG GGC ATT GAT AGT GCT AAA GTA AAA GTC ACC GTA AAA GCG 4224
Ile Asn Thr Gly Ile Asp Ser Ala Lys Val Lys Val Thr Val Lys Ala
        1395                1400                1405

GGT GGT GAC GAT CAA ATC TTT ACT GCT GAT AAT AGT ACC TAT GTT CCT 4272
Gly Gly Asp Asp Gln Ile Phe Thr Ala Asp Asn Ser Thr Tyr Val Pro
    1410                1415                1420

CAG CAA CCG GCA CCC AGT TTT GAG GAG ATG ATT TAT CAG TTC AAT AAC 4320
Gln Gln Pro Ala Pro Ser Phe Glu Glu Met Ile Tyr Gln Phe Asn Asn
1425                1430                1435                1440
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CTG ACA ATA GAT TGT AAG AAT TTA AAT TTC ATC GAC AAT CAG GCA CAT 4368
Leu Thr Ile Asp Cys Lys Asn Leu Asn Phe Ile Asp Asn Gln Ala His
                1445                1450                1455

ATT GAG ATT GAT TTC ACC GCT ACG GCA CAA GAT GGC CGA TTC TTG GGT 4416
Ile Glu Ile Asp Phe Thr Ala Thr Ala Gln Asp Gly Arg Phe Leu Gly
            1460                1465                1470

GCA GAA ACT TTT ATT ATC CCG GTA ACT AAA AAA GTT CTC GGT ACT GAG 4464
Ala Glu Thr Phe Ile Ile Pro Val Thr Lys Lys Val Leu Gly Thr Glu
        1475                1480                1485

AAC GTG ATT GCG TTA TAT AGC GAA AAT AAC GGT GTT CAA TAT ATG CAA 4512
Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly Val Gln Tyr Met Gln
    1490                1495                1500

ATT GGC GCA TAT CGT ACC CGT TTG AAT ACG TTA TTC GCT CAA CAG TTG 4560
Ile Gly Ala Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Gln Gln Leu
1505                1510                1515                1520

GTT AGC CGT GCT AAT CGT GGC ATT GAT GCA GTG CTC AGT ATG GAA ACT 4608
Val Ser Arg Ala Asn Arg Gly Ile Asp Ala Val Leu Ser Met Glu Thr
                1525                1530                1535

CAG AAT ATT CAG GAA CCG CAA TTA GGA GCG GGC ACA TAT GTG CAG CTT 4656
Gln Asn Ile Gln Glu Pro Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu
            1540                1545                1550

GTG TTG GAT AAA TAT GAT GAG TCT ATT CAT GGC ACT AAT AAA AGC TTT 4704
Val Leu Asp Lys Tyr Asp Glu Ser Ile His Gly Thr Asn Lys Ser Phe
        1555                1560                1565

GCT ATT GAA TAT GTT GAT ATA TTT AAA GAG AAC GAT AGT TTT GTG ATT 4752
Ala Ile Glu Tyr Val Asp Ile Phe Lys Glu Asn Asp Ser Phe Val Ile
    1570                1575                1580

TAT CAA GGA GAA CTT AGC GAA ACA AGT CAA ACT GTT GTG AAA GTT TTC 4800
Tyr Gln Gly Glu Leu Ser Glu Thr Ser Gln Thr Val Val Lys Val Phe
1585                1590                1595                1600

TTA TCC TAT TTT ATA GAG GCG ACT GGA AAT AAG AAC CAC TTA TGG GTA 4848
Leu Ser Tyr Phe Ile Glu Ala Thr Gly Asn Lys Asn His Leu Trp Val
                1605                1610                1615

CGT GCT AAA TAC CAA AAG GAA ACG ACT GAT AAG ATC TTG TTC GAC CGT 4896
Arg Ala Lys Tyr Gln Lys Glu Thr Thr Asp Lys Ile Leu Phe Asp Arg
            1620                1625                1630

ACT GAT GAG AAA GAT CCG CAC GGT TGG TTT CTC AGC GAC GAT CAC AAG 4944
Thr Asp Glu Lys Asp Pro His Gly Trp Phe Leu Ser Asp Asp His Lys
        1635                1640                1645

ACC TTT AGT GGT CTC TCT TCC GCA CAG GCA TTA AAG AAC GAC AGT GAA 4992
Thr Phe Ser Gly Leu Ser Ser Ala Gln Ala Leu Lys Asn Asp Ser Glu
    1650                1655                1660

CCG ATG GAT TTC TCT GGC GCC AAT GCT CTC TAT TTC TGG GAA CTG TTC 5040
Pro Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe
1665                1670                1675                1680

TAT TAC ACG CCG ATG ATG ATG GCT CAT CGT TTG TTG CAG GAA CAG AAT 5088
Tyr Tyr Thr Pro Met Met Met Ala His Arg Leu Leu Gln Glu Gln Asn
                1685                1690                1695

TTT GAT GCG GCG AAC CAT TGG TTC CGT TAT GTC TGG AGT CCA TCC GGT 5136
Phe Asp Ala Ala Asn His Trp Phe Arg Tyr Val Trp Ser Pro Ser Gly
            1700                1705                1710

TAT ATC GTT GAT GGT AAA ATT GCT ATC TAC CAC TGG AAC GTG CGA CCG 5184
Tyr Ile Val Asp Gly Lys Ile Ala Ile Tyr His Trp Asn Val Arg Pro
        1715                1720                1725
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CTG GAA GAA GAC ACC AGT TGG AAT GCA CAA CAA CTG GAC TCC ACC GAT 5232
Leu Glu Glu Asp Thr Ser Trp Asn Ala Gln Gln Leu Asp Ser Thr Asp
    1730                1735                1740

CCA GAT GCT GTA GCC CAA GAT GAT CCG ATG CAC TAC AAG GTG GCT ACC 5280
Pro Asp Ala Val Ala Gln Asp Asp Pro Met His Tyr Lys Val Ala Thr
1745                1750                1755                1760

TTT ATG GCG ACG TTG GAT CTG CTA ATG GCC CGT GGT GAT GCT GCT TAC 5328
Phe Met Ala Thr Leu Asp Leu Leu Met Ala Arg Gly Asp Ala Ala Tyr
                1765                1770                1775

CGC CAG TTA GAG CGT GAT ACG TTG GCT GAA GCT AAA ATG TGG TAT ACA 5376
Arg Gln Leu Glu Arg Asp Thr Leu Ala Glu Ala Lys Met Trp Tyr Thr
            1780                1785                1790

CAG GCG CTT AAT CTG TTG GGT GAT GAG CCA CAA GTG ATG CTG AGT ACG 5424
Gln Ala Leu Asn Leu Leu Gly Asp Glu Pro Gln Val Met Leu Ser Thr
        1795                1800                1805

ACT TGG GCT AAT CCA ACA TTG GGT AAT GCT GCT TCA AAA ACC ACA CAG 5472
Thr Trp Ala Asn Pro Thr Leu Gly Asn Ala Ala Ser Lys Thr Thr Gln
    1810                1815                1820

CAG GTT CGT CAG CAA GTG CTT ACC CAG TTG CGT CTC AAT AGC AGG GTA 5520
Gln Val Arg Gln Gln Val Leu Thr Gln Leu Arg Leu Asn Ser Arg Val
1825                1830                1835                1840

AAA ACC CCG TTG   5532
Lys Thr Pro Leu
            1844
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(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1844 amino acids

(B) TYPE: amino acids

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53 ($TcbA_{ii}$):

| Features | From | To | Description |
|---|---|---|---|
| Peptide | 1 | 1844 | $TcbA_{ii}$ peptide |
| Fragment | 1 | 11 | (SEQ ID NO:1) |
| Fragment | 978 | 990 | (SEQ ID NO:23) |
| Fragment | 1387 | 1401 | (SEQ ID NO:22) |
| Fragment | 1484 | 1505 | (SEQ ID NO:24) |
| Fragment | 1527 | 1552 | (SEQ ID NO:21) |

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Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala Asp Asn Tyr
 1               5                  10                  15

Ala Ala Pro Gly Ser Val Ala Ser Met Phe Ser Pro Ala Ala Tyr Leu
            20                  25                  30

Thr Glu Leu Tyr Arg Glu Ala Lys Asn Leu His Asp Ser Ser Ser Ile
        35                  40                  45

Tyr Tyr Leu Asp Lys Arg Arg Pro Asp Leu Ala Ser Leu Met Leu Ser
    50                  55                  60

Gln Lys Asn Met Asp Glu Glu Ile Ser Thr Leu Ala Leu Ser Asn Glu
65                  70                  75                  80

Leu Cys Leu Ala Gly Ile Glu Thr Lys Thr Gly Lys Ser Gln Asp Glu
```

85 90 95

Val Met Asp Met Leu Ser Thr Tyr Arg Leu Ser Gly Glu Thr Pro Tyr
100 105 110

His His Ala Tyr Glu Thr Val Arg Glu Ile Val His Glu Arg Asp Pro
115 120 125

Gly Phe Arg His Leu Ser Gln Ala Pro Ile Val Ala Ala Lys Leu Asp
130 135 140

Pro Val Thr Leu Leu Gly Ile Ser Ser His Ile Ser Pro Glu Leu Tyr
145 150 155 160

Asn Leu Leu Ile Glu Glu Ile Pro Glu Lys Asp Glu Ala Ala Leu Asp
165 170 175

Thr Leu Tyr Lys Thr Asn Phe Gly Asp Ile Thr Thr Ala Gln Leu Met
180 185 190

Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr Gly Val Ser Pro Glu Asp Ile
195 200 205

Ala Tyr Val Thr Thr Ser Leu Ser His Val Gly Tyr Ser Ser Asp Ile
210 215 220

Leu Val Ile Pro Leu Val Asp Gly Val Gly Lys Met Glu Val Val Arg
225 230 235 240

Val Thr Arg Thr Pro Ser Asp Asn Tyr Thr Ser Gln Thr Asn Tyr Ile
245 250 255

Glu Leu Tyr Pro Gln Gly Gly Asp Asn Tyr Leu Ile Lys Tyr Asn Leu
260 265 270

Ser Asn Ser Phe Gly Leu Asp Asp Phe Tyr Leu Gln Tyr Lys Asp Gly
275 280 285

Ser Ala Asp Trp Thr Glu Ile Ala His Asn Pro Tyr Pro Asp Met Val
290 295 300

Ile Asn Gln Lys Tyr Glu Ser Gln Ala Thr Ile Lys Arg Ser Asp Ser
305 310 315 320

Asp Asn Ile Leu Ser Ile Gly Leu Gln Arg Trp His Ser Gly Ser Tyr
325 330 335

Asn Phe Ala Ala Ala Asn Phe Lys Ile Asp Gln Tyr Ser Pro Lys Ala
340 345 350

Phe Leu Leu Lys Met Asn Lys Ala Ile Arg Leu Leu Lys Ala Thr Gly
355 360 365

Leu Ser Phe Ala Thr Leu Glu Arg Ile Val Asp Ser Val Asn Ser Thr
370 375 380

Lys Ser Ile Thr Val Glu Val Leu Asn Lys Val Tyr Arg Val Lys Phe
385 390 395 400

Tyr Ile Asp Arg Tyr Gly Ile Ser Glu Glu Thr Ala Ala Ile Leu Ala
405 410 415

Asn Ile Asn Ile Ser Gln Gln Ala Val Gly Asn Gln Leu Ser Gln Phe
420 425 430

Glu Gln Leu Phe Asn His Pro Pro Leu Asn Gly Ile Arg Tyr Glu Ile
435 440 445

Ser Glu Asp Asn Ser Lys His Leu Pro Asn Pro Asp Leu Asn Leu Lys
450 455 460

Pro Asp Ser Thr Gly Asp Asp Gln Arg Lys Ala Val Leu Lys Arg Ala
465 470 475 480

Phe Gln Val Asn Ala Ser Glu Leu Tyr Gln Met Leu Leu Ile Thr Asp
485 490 495

Arg Lys Glu Asp Gly Val Ile Lys Asn Asn Leu Glu Asn Leu Ser Asp
500 505 510

Leu Tyr Leu Val Ser Leu Leu Ala Gln Ile His Asn Leu Thr Ile Ala
515 520 525

Glu Leu Asn Ile Leu Leu Val Ile Cys Gly Tyr Gly Asp Thr Asn Ile
530 535 540

Tyr Gln Ile Thr Asp Asp Asn Leu Ala Lys Ile Val Glu Thr Leu Leu
545 550 555 560

Trp Ile Thr Gln Trp Leu Lys Thr Gln Lys Trp Thr Val Thr Asp Leu
565 570 575

Phe Leu Met Thr Thr Ala Thr Tyr Ser Thr Thr Leu Thr Pro Glu Ile
580 585 590

Ser Asn Leu Thr Ala Thr Leu Ser Ser Thr Leu His Gly Lys Glu Ser
595 600 605

Leu Ile Gly Glu Asp Leu Lys Arg Ala Met Ala Pro Cys Phe Thr Ser
610 615 620

Ala Leu His Leu Thr Ser Gln Glu Val Ala Tyr Asp Leu Leu Leu Trp
625 630 635 640

Ile Asp Gln Ile Gln Pro Ala Gln Ile Thr Val Asp Gly Phe Trp Glu
645 650 655

Glu Val Gln Thr Thr Pro Thr Ser Leu Lys Val Ile Thr Phe Ala Gln
660 665 670

Val Leu Ala Gln Leu Ser Leu Ile Tyr Arg Arg Ile Gly Leu Ser Glu
675 680 685

Thr Glu Leu Ser Leu Ile Val Thr Gln Ser Ser Leu Leu Val Ala Gly
690 695 700

Lys Ser Ile Leu Asp His Gly Leu Leu Thr Leu Met Ala Leu Glu Gly
705 710 715 720

Phe His Thr Trp Val Asn Gly Leu Gly Gln His Ala Ser Leu Ile Leu
725 730 735

Ala Ala Leu Lys Asp Gly Ala Leu Thr Val Thr Asp Val Ala Gln Ala
740 745 750

Met Asn Lys Glu Glu Ser Leu Leu Gln Met Ala Ala Asn Gln Val Glu
755 760 765

Lys Asp Leu Thr Lys Leu Thr Ser Trp Thr Gln Ile Asp Ala Ile Leu
770 775 780

Gln Trp Leu Gln Met Ser Ser Ala Leu Ala Val Ser Pro Leu Asp Leu
785 790 795 800

Ala Gly Met Met Ala Leu Lys Tyr Gly Ile Asp His Asn Tyr Ala Ala
805 810 815

Trp Gln Ala Ala Ala Ala Ala Leu Met Ala Asp His Ala Asn Gln Ala
820 825 830

Gln Lys Lys Leu Asp Glu Thr Phe Ser Lys Ala Leu Cys Asn Tyr Tyr
835 840 845

Ile Asn Ala Val Val Asp Ser Ala Ala Gly Val Arg Asp Arg Asn Gly
350 855 860

Leu Tyr Thr Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Asp Val Ile
865 870 875 880

Thr Ser Arg Ile Ala Glu Ala Ile Ala Gly Ile Gln Leu Tyr Val Asn
885 890 895

Arg Ala Leu Asn Arg Asp Glu Gly Gln Leu Ala Ser Asp Val Ser Thr
900 905 910

Arg Gln Phe Phe Thr Asp Trp Glu Arg Tyr Asn Lys Arg Tyr Ser Thr
915 920 925

Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro Glu Asn Tyr Val Asp
930 935 940

Pro Thr Gln Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln
945 950 955 960

Ser Ile Asn Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe
965 970 975

Lys Thr Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile
980 985 990

Ser Ala Tyr His Asp Asn Val Asn Val Asp Gln Gly Leu Thr Tyr Phe
995 1000 1005

Ile Gly Ile Asp Gln Ala Ala Pro Gly Thr Tyr Tyr Trp Arg Ser Val
1010 1015 1020

Asp His Ser Lys Cys Glu Asn Gly Lys Phe Ala Ala Asn Ala Trp Gly
1025 1030 1035 1040

Glu Trp Asn Lys Ile Thr Cys Ala Val Asn Pro Trp Lys Asn Ile Ile
1045 1050 1055

Arg Pro Val Val Tyr Met Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln
1060 1065 1070

Gln Ser Lys Lys Ser Asp Asp Gly Lys Thr Thr Ile Tyr Gln Tyr Asn
1075 1080 1085

Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Ser Trp Asn Thr Pro Phe
1090 1095 1100

Thr Phe Asp Val Thr Glu Lys Val Lys Asn Tyr Thr Ser Ser Thr Asp
1105 1110 1115 1120

Ala Ala Glu Ser Leu Gly Leu Tyr Cys Thr Gly Tyr Gln Gly Glu Asp
1125 1130 1135

Thr Leu Leu Val Met Phe Tyr Ser Met Gln Ser Ser Tyr Ser Ser Tyr
1140 1145 1150

Thr Asp Asn Asn Ala Pro Val Thr Gly Leu Tyr Ile Phe Ala Asp Met
1155 1160 1165

Ser Ser Asp Asn Met Thr Asn Ala Gln Ala Thr Asn Tyr Trp Asn Asn
1170 1175 1180

Ser Tyr Pro Gln Phe Asp Thr Val Met Ala Asp Pro Asp Ser Asp Asn
1185 1190 1195 1200

Lys Lys Val Ile Thr Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr
1205 1210 1215

Glu Ile Pro Ser Ser Val Thr Ser Asn Ser Asn Tyr Ser Trp Gly Asp

```
                1220                1225                1230
His Ser Leu Thr Met Leu Tyr Gly Gly Ser Val Pro Asn Ile Thr Phe
        1235                1240                1245

Glu Ser Ala Ala Glu Asp Leu Arg Leu Ser Thr Asn Met Ala Leu Ser
    1250                1255                1260

Ile Ile His Asn Gly Tyr Ala Gly Thr Arg Arg Ile Gln Cys Asn Leu
1265                1270                1275                1280

Met Lys Gln Tyr Ala Ser Leu Gly Asp Lys Phe Ile Ile Tyr Asp Ser
                1285                1290                1295

Ser Phe Asp Asp Ala Asn Arg Phe Asn Leu Val Pro Leu Phe Lys Phe
            1300                1305                1310

Gly Lys Asp Glu Asn Ser Asp Asp Ser Ile Cys Ile Tyr Asn Glu Asn
        1315                1320                1325

Pro Ser Ser Glu Asp Lys Lys Trp Tyr Phe Ser Ser Lys Asp Asp Asn
    1330                1335                1340

Lys Thr Ala Asp Tyr Asn Gly Gly Thr Gln Cys Ile Asp Ala Gly Thr
1345                1350                1355                1360

Ser Asn Lys Asp Phe Tyr Tyr Asn Leu Gln Glu Ile Glu Val Ile Ser
                1365                1370                1375

Val Thr Gly Gly Tyr Trp Ser Ser Tyr Lys Ile Ser Asn Pro Ile Asn
            1380                1385                1390

Ile Asn Thr Gly Ile Asp Ser Ala Lys Val Lys Val Thr Val Lys Ala
        1395                1400                1405

Gly Gly Asp Asp Gln Ile Phe Thr Ala Asp Asn Ser Thr Tyr Val Pro
    1410                1415                1420

Gln Gln Pro Ala Pro Ser Phe Glu Glu Met Ile Tyr Gln Phe Asn Asn
1425                1430                1435                1440

Leu Thr Ile Asp Cys Lys Asn Leu Asn Phe Ile Asp Asn Gln Ala His
                1445                1450                1455

Ile Glu Ile Asp Phe Thr Ala Thr Ala Gln Asp Gly Arg Phe Leu Gly
            1460                1465                1470

Ala Glu Thr Phe Ile Ile Pro Val Thr Lys Lys Val Leu Gly Thr Glu
        1475                1480                1485

Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly Val Gln Tyr Met Gln
    1490                1495                1500

Ile Gly Ala Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Gln Gln Leu
1505                1510                1515                1520

Val Ser Arg Ala Asn Arg Gly Ile Asp Ala Val Leu Ser Met Glu Thr
                1525                1530                1535

Gln Asn Ile Gln Glu Pro Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu
            1540                1545                1550

Val Leu Asp Lys Tyr Asp Glu Ser Ile His Gly Thr Asn Lys Ser Phe
        1555                1560                1565

Ala Ile Glu Tyr Val Asp Ile Phe Lys Glu Asn Asp Ser Phe Val Ile
    1570                1575                1580

Tyr Gln Gly Glu Leu Ser Glu Thr Ser Gln Thr Val Val Lys Val Phe
1585                1590                1595                1600
```

```
Leu Ser Tyr Phe Ile Glu Ala Thr Gly Asn Lys Asn His Leu Trp Val
                1605                1610                1615

Arg Ala Lys Tyr Gln Lys Glu Thr Thr Asp Lys Ile Leu Phe Asp Arg
            1620                1625                1630

Thr Asp Glu Lys Asp Pro His Gly Trp Phe Leu Ser Asp Asp His Lys
        1635                1640                1645

Thr Phe Ser Gly Leu Ser Ser Ala Gln Ala Leu Lys Asn Asp Ser Glu
    1650                1655                1660

Pro Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe
1665                1670                1675                1680

Tyr Tyr Thr Pro Met Met Met Ala His Arg Leu Leu Gln Glu Gln Asn
                1685                1690                1695

Phe Asp Ala Ala Asn His Trp Phe Arg Tyr Val Trp Ser Pro Ser Gly
            1700                1705                1710

Tyr Ile Val Asp Gly Lys Ile Ala Ile Tyr His Trp Asn Val Arg Pro
        1715                1720                1725

Leu Glu Glu Asp Thr Ser Trp Asn Ala Gln Gln Leu Asp Ser Thr Asp
    1730                1735                1740

Pro Asp Ala Val Ala Gln Asp Asp Pro Met His Tyr Lys Val Ala Thr
1745                1750                1755                1760

Phe Met Ala Thr Leu Asp Leu Leu Met Ala Arg Gly Asp Ala Ala Tyr
                1765                1770                1775

Arg Gln Leu Glu Arg Asp Thr Leu Ala Glu Ala Lys Met Trp Tyr Thr
            1780                1785                1790

Gln Ala Leu Asn Leu Leu Gly Asp Glu Pro Gln Val Met Leu Ser Thr
        1795                1800                1805

Thr Trp Ala Asn Pro Thr Leu Gly Asn Ala Ala Ser Lys Thr Thr Gln
    1810                1815                1820

Gln Val Arg Gln Gln Val Leu Thr Gln Leu Arg Leu Asn Ser Arg Val
1825                1830                1835                1840

Lys Thr Pro Leu
            1844
```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1722 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54 ($TcbA_{iii}$ coding region):

```
CTA GGA ACA GCC AAT TCC CTG ACC GCT TTA TTC CTG CCG CAG GAA AAT 48
Leu Gly Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn
 1               5                  10                  15

AGC AAG CTC AAA GGC TAC TGG CGG ACA CTG GCG CAG CGT ATG TTT AAT 96
Ser Lys Leu Lys Gly Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn
            20                  25                  30
```

```
TTA CGT CAT AA[redacted]TG TCG ATT GAC GGC CAG CCG CTC [redacted]TTG CCG CTG 144
Leu Arg His As[redacted]eu Ser Ile Asp Gly Gln Pro Leu [redacted] Leu Pro Leu
          35                  40                  45

TAT GCT AAA CCG GCT GAT CCA AAA GCT TTA CTG AGT GCG GCG GTT TCA 192
Tyr Ala Lys Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser
      50                  55                  60

GCT TCT CAA GGG GGA GCC GAC TTG CCG AAG GCG CCG CTG ACT ATT CAC 240
Ala Ser Gln Gly Gly Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His
65                  70                  75                  80

CGC TTC CCT CAA ATG CTA GAA GGG GCA CGG GGC TTG GTT AAC CAG CTT 288
Arg Phe Pro Gln Met Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu
                85                  90                  95

ATA CAG TTC GGT AGT TCA CTA TTG GGG TAC AGT GAG CGT CAG GAT GCG 336
Ile Gln Phe Gly Ser Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala
           100                 105                 110

GAA GCT ATG AGT CAA CTA CTG CAA ACC CAA GCC AGC GAG TTA ATA CTG 384
Glu Ala Met Ser Gln Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu
      115                 120                 125

ACC AGT ATT CGT ATG CAG GAT AAC CAA TTG GCA GAG CTG GAT TCG GAA 432
Thr Ser Ile Arg Met Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu
  130                 135                 140

AAA ACC GCC TTG CAA GTC TCT TTA GCT GGA GTG CAA CAA CGG TTT GAC 480
Lys Thr Ala Leu Gln Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp
145                 150                 155                 160

AGC TAT AGC CAA CTG TAT GAG GAG AAC ATC AAC GCA GGT GAG CAG CGA 528
Ser Tyr Ser Gln Leu Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg
                165                 170                 175

GCG CTG GCG TTA CGC TCA GAA TCT GCT ATT GAG TCT CAG GGA GCG CAG 576
Ala Leu Ala Leu Arg Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln
           180                 185                 190

ATT TCC CGT ATG GCA GGC GCG GGT GTT GAT ATG GCA CCA AAT ATC TTC 624
Ile Ser Arg Met Ala Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe
      195                 200                 205

GGC CTG GCT GAT GGC GGC ATG CAT TAT GGT GCT ATT GCC TAT GCC ATC 672
Gly Leu Ala Asp Gly Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile
  210                 215                 220

GCT GAC GGT ATT GAG TTG AGT GCT TCT GCC AAG ATG GTT GAT GCG GAG 720
Ala Asp Gly Ile Glu Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu
225                 230                 235                 240

AAA GTT GCT CAG TCG GAA ATA TAT CGC CGT CGC CGT CAA GAA TGG AAA 768
Lys Val Ala Gln Ser Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys
                245                 250                 255

ATT CAG CGT GAC AAC GCA CAA GCG GAG ATT AAC CAG TTA AAC GCG CAA 816
Ile Gln Arg Asp Asn Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln
           260                 265                 270

CTG GAA TCA CTG TCT ATT CGC CGT GAA GCC GCT GAA ATG CAA AAA GAG 864
Leu Glu Ser Leu Ser Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu
      275                 280                 285

TAC CTG AAA ACC CAG CAA GCT CAG GCG CAG GCA CAA CTT ACT TTC TTA 912
Tyr Leu Lys Thr Gln Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu
  290                 295                 300

AGA AGC AAA TTC AGT AAT CAA GCG TTA TAT AGT TGG TTA CGA GGG CGT 960
Arg Ser Lys Phe Ser Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg
305                 310                 315                 320
```

```
TTG TCA GGT ATT TAT TTC CAG TTC TAT GAC TTG GCC GTA TCA CGT TGC 1008
Leu Ser Gly Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys
            325                 330                 335

CTG ATG GCA GAG CAA TCC TAT CAA TGG GAA GCT AAT GAT AAT TCC ATT 1056
Leu Met Ala Glu Gln Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile
        340                 345                 350

AGC TTT GTC AAA CCG GGT GCA TGG CAA GGA ACT TAC GCC GGC TTA TTG 1104
Ser Phe Val Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
    355                 360                 365

TGT GGA GAA GCT TTG ATA CAA AAT CTG GCA CAA ATG GAA GAG GCA TAT 1152
Cys Gly Glu Ala Leu Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr
370                 375                 380

CTG AAA TGG GAA TCT CGC GCT TTG GAA GTA GAA CGC ACG GTT TCA TTG 1200
Leu Lys Trp Glu Ser Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
385                 390                 395                 400

GCA GTG GTT TAT GAT TCA CTG GAA GGT AAT GAT CGT TTT AAT TTA GCG 1248
Ala Val Val Tyr Asp Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala
            405                 410                 415

GAA CAA ATA CCT GCA TTA TTG GAT AAG GGG GAG GGA ACA GCA GGA ACT 1296
Glu Gln Ile Pro Ala Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr
        420                 425                 430

AAA GAA AAT GGG TTA TCA TTG GCT AAT GCT ATC CTG TCA GCT TCG GTC 1344
Lys Glu Asn Gly Leu Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val
    435                 440                 445

AAA TTG TCC GAC TTG AAA CTG GGA ACG GAT TAT CCA GAC AGT ATC GTT 1392
Lys Leu Ser Asp Leu Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val
450                 455                 460

GGT AGC AAC AAG GTT CGT CGT ATT AAG CAA ATC AGT GTT TCG CTA CCT 1440
Gly Ser Asn Lys Val Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro
465                 470                 475                 480

GCA TTG GTT GGG CCT TAT CAG GAT GTT CAG GCT ATG CTC AGC TAT GGT 1488
Ala Leu Val Gly Pro Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly
            485                 490                 495

GGC AGT ACT CAA TTG CCG AAA GGT TGT TCA GCG TTG GCT GTG TCT CAT 1536
Gly Ser Thr Gln Leu Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His
        500                 505                 510

GGT ACC AAT GAT AGT GGT CAG TTC CAG TTG GAT TTC AAT GAC GGC AAA 1584
Gly Thr Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys
    515                 520                 525

TAC CTG CCA TTT GAA GGT ATT GCT CTT GAT GAT CAG GGT ACA CTG AAT 1632
Tyr Leu Pro Phe Glu Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn
530                 535                 540

CTT CAA TTT CCG AAT GCT ACC GAC AAG CAG AAA GCA ATA TTG CAA ACT 1680
Leu Gln Phe Pro Asn Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr
545                 550                 555                 560

ATG AGC GAT ATT ATT TTG CAT ATT CGT TAT ACC ATC CGT TAA      1722
Met Ser Asp Ile Ile Leu His Ile Arg Tyr Thr Ile Arg ...
            565                 570                 573
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 573 amino acids

(B) TYPE: amino acids

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55 (TcbA$_{iii}$):

```
Leu Gly Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn
 1               5                  10                  15

Ser Lys Leu Lys Gly Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn
            20                  25                  30

Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu
        35                  40                  45

Tyr Ala Lys Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser
    50                  55                  60

Ala Ser Gln Gly Gly Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His
65                  70                  75                  80

Arg Phe Pro Gln Met Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu
                85                  90                  95

Ile Gln Phe Gly Ser Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala
            100                 105                 110

Glu Ala Met Ser Gln Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu
        115                 120                 125

Thr Ser Ile Arg Met Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu
    130                 135                 140

Lys Thr Ala Leu Gln Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp
145                 150                 155                 160

Ser Tyr Ser Gln Leu Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg
                165                 170                 175

Ala Leu Ala Leu Arg Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln
            180                 185                 190

Ile Ser Arg Met Ala Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe
        195                 200                 205

Gly Leu Ala Asp Gly Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile
    210                 215                 220

Ala Asp Gly Ile Glu Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu
225                 230                 235                 240

Lys Val Ala Gln Ser Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys
                245                 250                 255

Ile Gln Arg Asp Asn Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln
            260                 265                 270

Leu Glu Ser Leu Ser Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu
        275                 280                 285

Tyr Leu Lys Thr Gln Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu
    290                 295                 300

Arg Ser Lys Phe Ser Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg
305                 310                 315                 320

Leu Ser Gly Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys
                325                 330                 335
```

```
Leu Met Ala Glu Gln Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile
            340                 345                 350

Ser Phe Val Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
        355                 360                 365

Cys Gly Glu Ala Leu Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr
    370                 375                 380

Leu Lys Trp Glu Ser Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
385                 390                 395                 400

Ala Val Val Tyr Asp Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala
                405                 410                 415

Glu Gln Ile Pro Ala Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr
            420                 425                 430

Lys Glu Asn Gly Leu Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val
        435                 440                 445

Lys Leu Ser Asp Leu Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val
    450                 455                 460

Gly Ser Asn Lys Val Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro
465                 470                 475                 480

Ala Leu Val Gly Pro Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly
                485                 490                 495

Gly Ser Thr Gln Leu Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His
            500                 505                 510

Gly Thr Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys
        515                 520                 525

Tyr Leu Pro Phe Glu Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn
    530                 535                 540

Leu Gln Phe Pro Asn Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr
545                 550                 555                 560

Met Ser Asp Ile Ile Leu His Ile Arg Tyr Thr Ile Arg ...
                565                 570         573
```

(2) INFORMATION FOR SEQ ID NO:56

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2898 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56 (*tccA*)

```
1   ATG AAT CAA CTC GCC AGT CCC CTG ATT TCC CGC ACC GAA GAG ATC CAC   48
1   Met Asn Gln Leu Ala Ser Pro Leu Ile Ser Arg Thr Glu Glu Ile His   16

49  AAC TTA CCC GGT AAA TTG ACC GAT CTT GGT TAT ACC TCA GTG TTT GAT   96
17  Asn Leu Pro Gly Lys Leu Thr Asp Leu Gly Tyr Thr Ser Val Phe Asp   32

97  GTG GTA CGT ATG CCG CGT GAG CGT TTT ATT CGT GAG CAT CGT GCT GAT   144
```

```
     33  Val Val Arg Met Pro Arg Glu Arg Phe Ile Arg Glu His Arg Ala Asp   48

    145  CTC GGG CGC AGT GCT GAA AAA ATG TAT GAC CTG GCA GTG GGC TAT GCT   192
     49  Leu Gly Arg Ser Ala Glu Lys Met Tyr Asp Leu Ala Val Gly Tyr Ala    64

    193  CAT CAG GTG TTA CAC CAT TTT CGC CGT AAT TCT CTT AGT GAA GCT GTT   240
     65  His Gln Val Leu His His Phe Arg Arg Asn Ser Leu Ser Glu Ala Val    80

    241  CAG TTT GGC TTG AGA AGT CCG TTC TCC GTA TCA GGC CCG GAT TAC GCC   288
     81  Gln Phe Gly Leu Arg Ser Pro Phe Ser Val Ser Gly Pro Asp Tyr Ala    96

    289  AAT CAG TTT CTT GAT GCA AAC ACG GGT TGG AAA GAT AAA GCA CCA AGT   336
     97  Asn Gln Phe Leu Asp Ala Asn Thr Gly Trp Lys Asp Lys Ala Pro Ser   112

    337  GGA TCA CCG GAA GCC AAT GAT GCG CCG GTA GCC TAT CTG ACT CAT ATT   384
    113  Gly Ser Pro Glu Ala Asn Asp Ala Pro Val Ala Tyr Leu Thr His Ile   128

    385  TAT CAA TTG GCC CTT GAA CAG GAA AAG AAT GGC GCC ACT ACC ATT ATG   432
    129  Tyr Gln Leu Ala Leu Glu Gln Glu Lys Asn Gly Ala Thr Thr Ile Met   144

    433  AAT ACG CTG GCG GAG CGT CGC CCC GAT CTG GGT GCT TTG TTA ATT AAT   480
    145  Asn Thr Leu Ala Glu Arg Arg Pro Asp Leu Gly Ala Leu Leu Ile Asn   160

    481  GAT AAA GCA ATC AAT GAG GTG ATA CCG CAA TTG CAG TTG GTC AAT GAA   528
    161  Asp Lys Ala Ile Asn Glu Val Ile Pro Gln Leu Gln Leu Val Asn Glu   176

    529  ATT CTG TCC AAA GCT ATT CAG AAG AAA CTG AGT TTG ACT GAT CTG GAA   576
    177  Ile Leu Ser Lys Ala Ile Gln Lys Lys Leu Ser Leu Thr Asp Leu Glu   192

    577  GCG GTA AAC GCC AGA CTT TCC ACT ACC CGT TAC CCG AAT AAT CTG CCG   624
    193  Ala Val Asn Ala Arg Leu Ser Thr Thr Arg Tyr Pro Asn Asn Leu Pro   208

    625  TAT CAT TAT GGT CAT CAG CAG ATT CAG ACA GCT CAA TCG GTA TTG GGT   672
    209  Tyr His Tyr Gly His Gln Gln Ile Gln Thr Ala Gln Ser Val Leu Gly   224

    673  ACT ACG TTG CAA GAT ATC ACT TTG CCA CAG ACG CTG GAT CTG CCG CAA   720
    225  Thr Thr Leu Gln Asp Ile Thr Leu Pro Gln Thr Leu Asp Leu Pro Gln   240

    721  AAC TTC TGG GCA ACA GCA AAA GGA AAA CTG AGC GAT ACG ACT GCC AGT   768
    241  Asn Phe Trp Ala Thr Ala Lys Gly Lys Leu Ser Asp Thr Thr Ala Ser   256

    769  GCT TTG ACC CGA CTG CAA ATC ATG GCG AGT CAG TTT TCG CCA GAG CAG   816
    257  Ala Leu Thr Arg Leu Gln Ile Met Ala Ser Gln Phe Ser Pro Glu Gln   272

    817  CAG AAA ATC ATT ACG GAG ACT GTC GGT CAG GAT TTC TAT CAG CTT AAC   864
    273  Gln Lys Ile Ile Thr Glu Thr Val Gly Gln Asp Phe Tyr Gln Leu Asn   288

    865  TAT GGT GAC AGT TCG CTT ACT GTG AAT AGT TTC AGC GAC ATG ACC ATA   912
    289  Tyr Gly Asp Ser Ser Leu Thr Val Asn Ser Phe Ser Asp Met Thr Ile   304
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913  ATG ACT GAT CGA ACA AGT TTG ACT GTA CCC CAG GTA GAA CTG ATG TTG   960
305  Met Thr Asp Arg Thr Ser Leu Thr Val Pro Gln Val Glu Leu Met Leu   320

961  TGT TCA ACT GTC GGA GGT TCT ACG GTT GTT AAG TCT GAT AAT GTG AGT  1008
321  Cys Ser Thr Val Gly Gly Ser Thr Val Val Lys Ser Asp Asn Val Ser   336

1009  TCT GGT GAC ACG ACA GCG ACG CCA TTT GCG TAT GGC GCC CGC TTT ATT  1056
337  Ser Gly Asp Thr Thr Ala Thr Pro Phe Ala Tyr Gly Ala Arg Phe Ile   352

1057  CAT GCC GGT AAG CCG GAG GCG ATT ACC CTG AGT CGC AGT GGT GCG GAG  1104
353  His Ala Gly Lys Pro Glu Ala Ile Thr Leu Ser Arg Ser Gly Ala Glu   368

1105  GCG CAT TTT GCT CTG ACG GTT AAC AAT CTG ACA GAT GAC AAG TTG GAC  1152
369  Ala His Phe Ala Leu Thr Val Asn Asn Leu Thr Asp Asp Lys Leu Asp   384

1153  CGT ATT AAC CGC ACA GTG CGC CTG CAA AAA TGG CTG AAT CTG CCT TAT  1200
385  Arg Ile Asn Arg Thr Val Arg Leu Gln Lys Trp Leu Asn Leu Pro Tyr   400

1201  GAG GAT ATT GAC CTG TTA GTG ACT TCT GCT ATG GAT GCG GAA ACA GGA  1248
401  Glu Asp Ile Asp Leu Leu Val Thr Ser Ala Met Asp Ala Glu Thr Gly   416

1249  AAT ACC GCG CTG TCG ATG AAC GAC AAT ACG CTG CGT ATG TTG GGA GTG  1296
417  Asn Thr Ala Leu Ser Met Asn Asp Asn Thr Leu Arg Met Leu Gly Val   432

1297  TTC AAA CAT TAT CAG GCG AAG TAT GGT GTT AGC GCT AAA CAA TTT GCT  1344
433  Phe Lys His Tyr Gln Ala Lys Tyr Gly Val Ser Ala Lys Gln Phe Ala   448

1345  GGC TGG CTG CGC GTA GTG GCC CCG TTT GCC ATT ACA CCG GCA ACG CCG  1392
449  Gly Trp Leu Arg Val Val Ala Pro Phe Ala Ile Thr Pro Ala Thr Pro   464

1393  TTT TTA GAC CAA GTG TTT AAC TCC GTC GGC ACC TTT GAT ACA CCG TTT  1440
465  Phe Leu Asp Gln Val Phe Asn Ser Val Gly Thr Phe Asp Thr Pro Phe   480

1441  GTG ATA GAT AAT CAG GAT TTT GTC TAT ACA TTG ACC ACC GGG GGC GAT  1488
481  Val Ile Asp Asn Gln Asp Phe Val Tyr Thr Leu Thr Thr Gly Gly Asp   496

1489  GGG GCG CGT GTT AAG CAT ATC AGC ACG GCA CTG GGC CTC AAT CAT CGT  1536
497  Gly Ala Arg Val Lys His Ile Ser Thr Ala Leu Gly Leu Asn His Arg   512

1537  CAG TTC CTG TTA TTG GCG GAT AAT ATT GCC CGT CAA CAG GGG AAT GTC  1584
513  Gln Phe Leu Leu Leu Ala Asp Asn Ile Ala Arg Gln Gln Gly Asn Val   528

1585  ACG CAA AGC ACA CTC AAC TGT AAT CTG TTT GTG GTG TCA GCT TTC TAC  1632
529  Thr Gln Ser Thr Leu Asn Cys Asn Leu Phe Val Val Ser Ala Phe Tyr   544

1633  CGT CTG GCT AAT TTG GCG CGC ACA TTG GGG ATA AAT CCA GAG TCT TTC  1680
545  Arg Leu Ala Asn Leu Ala Arg Thr Leu Gly Ile Asn Pro Glu Ser Phe   560

1681  TGT GCC TTG GTT GAT CGA TTA GAT GCA GGT ACA GGC ATC GTC TGG CAG  1728
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 561  Cys Ala Leu Val Asp Arg Leu Asp Ala Gly Thr Gly Ile Val Trp Gln   574

1729  CAA TTG GCA GGG AAA CCC ACA ATC ACG GTA CCA CAA AAA GAT TCC CCG  1776
 577  Gln Leu Ala Gly Lys Pro Thr Ile Thr Val Pro Gln Lys Asp Ser Pro   592

1777  CTG GCG GCG GAT ATT CTG AGT TTG CTG CAA GCG CTA AGT GCG ATT GCT
1824
 593  Leu Ala Ala Asp Ile Leu Ser Leu Leu Gln Ala Leu Ser Ala Ile Ala
608

1825  CAA TGG CAA CAA CAG CAC GAT TTA GAA TTT TCA GCA CTG CTT TTG CTG  1872
 609  Gln Trp Gln Gln Gln His Asp Leu Glu Phe Ser Ala Leu Leu Leu Leu   624

1873  TTG AGT GAC AAC CCT ATT TCT ACC TCG CAG GGC ACT GAC GAT CAA TTG  1920
 625  Leu Ser Asp Asn Pro Ile Ser Thr Ser Gln Gly Thr Asp Asp Gln Leu   640

1921  AAC TTT ATC CGT CAA GTG TGG CAG AAC CTA GGC AGT ACG TTT GTG GGT  1968
 641  Asn Phe Ile Arg Gln Val Trp Gln Asn Leu Gly Ser Thr Phe Val Gly   656

1969  GCA ACA TTG TTG TCC CGC AGT GGG GCA CCA TTA GTC GAT ACC AAC GGC  2016
 657  Ala Thr Leu Leu Ser Arg Ser Gly Ala Pro Leu Val Asp Thr Asn Gly   672

2017  CAC GCT ATT GAC TGG TTT GCT CTG CTC TCA GCA GGT AAT AGT CCG CTT  2064
 673  His Ala Ile Asp Trp Phe Ala Leu Leu Ser Ala Gly Asn Ser Pro Leu   688

2065  ATC GAT AAG GTT GGT CTG GTG ACT GAT GCT GGC ATA CAA AGT GTT ATA  2112
 689  Ile Asp Lys Val Gly Leu Val Thr Asp Ala Gly Ile Gln Ser Val Ile   704

2113  GCA ACG GTG GTC AAT ACA CAA AGC TTA TCT GAT GAA GAT AAG AAG CTG  2160
 705  Ala Thr Val Val Asn Thr Gln Ser Leu Ser Asp Glu Asp Lys Lys Leu   720

2161  GCA ATC ACT ACT CTG ACT AAT ACG TTG AAT CAG GTA CAG AAA ACT CAA  2208
 721  Ala Ile Thr Thr Leu Thr Asn Thr Leu Asn Gln Val Gln Lys Thr Gln   736

2209  CAG GGC GTG GCC GTC AGT CTG TTG GCG CAG ACT CTG AAC GTG AGT CAG  2256
 737  Gln Gly Val Ala Val Ser Leu Leu Ala Gln Thr Leu Asn Val Ser Gln   752

2257  TCA CTG CCT GCG TTA TTG TTG CGC TGG AGT GGA CAA ACA ACC TAC CAG  2304
 753  Ser Leu Pro Ala Leu Leu Leu Arg Trp Ser Gly Gln Thr Thr Tyr Gln   768

2305  TGG TTG AGT GCG ACT TGG GCA TTG AAG GAT GCC GTT AAG ACT GCC GCC  2352
 769  Trp Leu Ser Ala Thr Trp Ala Leu Lys Asp Ala Val Lys Thr Ala Ala   784

2353  GAT ATT CCC GCT GAC TAT CTG CGT CAA TTA CGT GAA GTG GTA CGC CGC  2400
 785  Asp Ile Pro Ala Asp Tyr Leu Arg Gln Leu Arg Glu Val Val Arg Arg   800

2401  TCC TTG TTG ACC CAA CAA TTC ACG CTG AGT CCT GCA ATG GTG CAA ACC  2448
 801  Ser Leu Leu Thr Gln Gln Phe Thr Leu Ser Pro Ala Met Val Gln Thr   816
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2449  TTG CTG GAC TAT CCA GCC TAT TTT GGC GCT TCC GCA GAA ACA GTG ACC  2496
 817  Leu Leu Asp Tyr Pro Ala Tyr Phe Gly Ala Ser Ala Glu Thr Val Thr  832

2497  GAT ATC AGT TTG TGG ATG CTT TAT ACC CTG AGC TGT TAT AGC GAT TTA  2544
 833  Asp Ile Ser Leu Trp Met Leu Tyr Thr Leu Ser Cys Tyr Ser Asp Leu  848

2545  TTG CTC CAA ATG GGT GAA GCT GGT GGT ACC GAA GAT GAT GTA CTG GCC  2592
 849  Leu Leu Gln Met Gly Glu Ala Gly Gly Thr Glu Asp Asp Val Leu Ala  864

2593  TAC TTA CGC ACA GCT AAT GCT ACC ACA CCG TTG AGC CAA TCT GAT GCT  2640
 865  Tyr Leu Arg Thr Ala Asn Ala Thr Thr Pro Leu Ser Gln Ser Asp Ala  880

2641  GCA CAG ACG TTG GCA ACG CTA TTG GGT TGG GAG GTT AAC GAG TTG CAA  2688
 881  Ala Gln Thr Leu Ala Thr Leu Leu Gly Trp Glu Val Asn Glu Leu Gln  896

2689  GCC GCT TGG TCG GTA TTG GGC GGG ATT GCC AAA ACC ACA CCG CAA CTG  2736
 897  Ala Ala Trp Ser Val Leu Gly Gly Ile Ala Lys Thr Thr Pro Gln Leu  912

2737  GAT GCG CTT CTG CGT TTG CAA CAG GCA CAG AAC CAA ACT GGT CTT GGC  2784
 913  Asp Ala Leu Leu Arg Leu Gln Gln Ala Gln Asn Gln Thr Gly Leu Gly  928

2785  GTT ACA CAG CAA CAG CAA GGC TAT CTC CTG AGT CGT GAC AGT GAT TAT  2832
 929  Val Thr Gln Gln Gln Gln Gly Tyr Leu Leu Ser Arg Asp Ser Asp Tyr  944

2833  ACC CTT TGG CAA AGC ACC GGT CAG GCG CTG GTG GCT GGC GTA TCC CAT  2880
 945  Thr Leu Trp Gln Ser Thr Gly Gln Ala Leu Val Ala Gly Val Ser His  960

2881  GTC AAG GGC AGT AAC TGA   2898
 961  Val Lys Gly Ser Asn End   966
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(2) INFORMATION FOR SEQ ID NO:57

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 965 amino acids

(B) TYPE: amino acid

(C) TOPOLOGY: linea
r

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57 (TccA peptide)

| Features | From | To | Description |
|---|---|---|---|
| | 1 | 10 | SEQ ID NO:8 |

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  1  Met Asn Gln Leu Ala Ser Pro Leu Ile Ser Arg Thr Glu Glu Ile His   16

 17  Asn Leu Pro Gly Lys Leu Thr Asp Leu Gly Tyr Thr Ser Val Phe Asp   32

 33  Val Val Arg Met Pro Arg Glu Arg Phe Ile Arg Glu His Arg Ala Asp   48

 49  Leu Gly Arg Ser Ala Glu Lys Met Tyr Asp Leu Ala Val Gly Tyr Ala   64

 65  His Gln Val Leu His His Phe Arg Arg Asn Ser Leu Ser Glu Ala Val   80

 81  Gln Phe Gly Leu Arg Ser Pro Phe Ser Val Ser Gly Pro Asp Tyr Ala   96
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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 97 | Asn | Gln | Phe | Leu | Asp | Ala | Asn | Thr | Gly | Trp | Lys | Asp | Lys | Ala | Pro | Ser | 112 |
| 113 | Gly | Ser | Pro | Glu | Ala | Asn | Asp | Ala | Pro | Val | Ala | Tyr | Leu | Thr | His | Ile | 128 |
| 129 | Tyr | Gln | Leu | Ala | Leu | Glu | Gln | Glu | Lys | Asn | Gly | Ala | Thr | Thr | Ile | Met | 144 |
| 145 | Asn | Thr | Leu | Ala | Glu | Arg | Arg | Pro | Asp | Leu | Gly | Ala | Leu | Leu | Ile | Asn | 160 |
| 161 | Asp | Lys | Ala | Ile | Asn | Glu | Val | Ile | Pro | Gln | Leu | Gln | Leu | Val | Asn | Glu | 176 |
| 177 | Ile | Leu | Ser | Lys | Ala | Ile | Gln | Lys | Lys | Leu | Ser | Leu | Thr | Asp | Leu | Glu | 192 |
| 193 | Ala | Val | Asn | Ala | Arg | Leu | Ser | Thr | Thr | Arg | Tyr | Pro | Asn | Asn | Leu | Pro | 208 |
| 209 | Tyr | His | Tyr | Gly | His | Gln | Gln | Ile | Gln | Thr | Ala | Gln | Ser | Val | Leu | Gly | 224 |
| 225 | Thr | Thr | Leu | Gln | Asp | Ile | Thr | Leu | Pro | Gln | Thr | Leu | Asp | Leu | Pro | Gln | 240 |
| 241 | Asn | Phe | Trp | Ala | Thr | Ala | Lys | Gly | Lys | Leu | Ser | Asp | Thr | Thr | Ala | Ser | 256 |
| 257 | Ala | Leu | Thr | Arg | Leu | Gln | Ile | Met | Ala | Ser | Gln | Phe | Ser | Pro | Glu | Gln | 272 |
| 273 | Gln | Lys | Ile | Ile | Thr | Glu | Thr | Val | Gly | Gln | Asp | Phe | Tyr | Gln | Leu | Asn | 288 |
| 289 | Tyr | Gly | Asp | Ser | Ser | Leu | Thr | Val | Asn | Ser | Phe | Ser | Asp | Met | Thr | Ile | 304 |
| 305 | Met | Thr | Asp | Arg | Thr | Ser | Leu | Thr | Val | Pro | Gln | Val | Glu | Leu | Met | Leu | 320 |
| 321 | Cys | Ser | Thr | Val | Gly | Gly | Ser | Thr | Val | Val | Lys | Ser | Asp | Asn | Val | Ser | 336 |
| 337 | Ser | Gly | Asp | Thr | Thr | Ala | Thr | Pro | Phe | Ala | Tyr | Gly | Ala | Arg | Phe | Ile | 352 |
| 353 | His | Ala | Gly | Lys | Pro | Glu | Ala | Ile | Thr | Leu | Ser | Arg | Ser | Gly | Ala | Glu | 368 |
| 369 | Ala | His | Phe | Ala | Leu | Thr | Val | Asn | Asn | Leu | Thr | Asp | Asp | Lys | Leu | Asp | 384 |
| 385 | Arg | Ile | Asn | Arg | Thr | Val | Arg | Leu | Gln | Lys | Trp | Leu | Asn | Leu | Pro | Tyr | 400 |
| 401 | Glu | Asp | Ile | Asp | Leu | Leu | Val | Thr | Ser | Ala | Met | Asp | Ala | Glu | Thr | Gly | 416 |
| 417 | Asn | Thr | Ala | Leu | Ser | Met | Asn | Asp | Asn | Thr | Leu | Arg | Met | Leu | Gly | Val | 432 |
| 433 | Phe | Lys | His | Tyr | Gln | Ala | Lys | Tyr | Gly | Val | Ser | Ala | Lys | Gln | Phe | Ala | 448 |
| 449 | Gly | Trp | Leu | Arg | Val | Val | Ala | Pro | Phe | Ala | Ile | Thr | Pro | Ala | Thr | Pro | 464 |
| 465 | Phe | Leu | Asp | Gln | Val | Phe | Asn | Ser | Val | Gly | Thr | Phe | Asp | Thr | Pro | Phe | 480 |
| 481 | Val | Ile | Asp | Asn | Gln | Asp | Phe | Val | Tyr | Thr | Leu | Thr | Thr | Gly | Gly | Asp | 496 |
| 497 | Gly | Ala | Arg | Val | Lys | His | Ile | Ser | Thr | Ala | Leu | Gly | Leu | Asn | His | Arg | 512 |
| 513 | Gln | Phe | Leu | Leu | Leu | Ala | Asp | Asn | Ile | Ala | Arg | Gln | Gln | Gly | Asn | Val | 528 |
| 529 | Thr | Gln | Ser | Thr | Leu | Asn | Cys | Asn | Leu | Phe | Val | Val | Ser | Ala | Phe | Tyr | 544 |
| 545 | Arg | Leu | Ala | Asn | Leu | Ala | Arg | Thr | Leu | Gly | Ile | Asn | Pro | Glu | Ser | Phe | 560 |
| 561 | Cys | Ala | Leu | Val | Asp | Arg | Leu | Asp | Ala | Gly | Thr | Gly | Ile | Val | Trp | Gln | 576 |
| 577 | Gln | Leu | Ala | Gly | Lys | Pro | Thr | Ile | Thr | Val | Pro | Gln | Lys | Asp | Ser | Pro | 592 |
| 593 | Leu | Ala | Ala | Asp | Ile | Leu | Ser | Leu | Leu | Gln | Ala | Leu | Ser | Ala | Ile | Ala | 608 |
| 609 | Gln | Trp | Gln | Gln | Gln | His | Asp | Leu | Glu | Phe | Ser | Ala | Leu | Leu | Leu | Leu | 624 |

625 Leu Ser Asp Asn Pro Ile Ser Thr Ser Gln Gly Thr Asp Asp Gln Leu 640

641 Asn Phe Ile Arg Gln Val Trp Gln Asn Leu Gly Ser Thr Phe Val Gly 656

657 Ala Thr Leu Leu Ser Arg Ser Gly Ala Pro Leu Val Asp Thr Asn Gly 672

673 His Ala Ile Asp Trp Phe Ala Leu Leu Ser Ala Gly Asn Ser Pro Leu 688

689 Ile Asp Lys Val Gly Leu Val Thr Asp Ala Gly Ile Gln Ser Val Ile 704

705 Ala Thr Val Val Asn Thr Gln Ser Leu Ser Asp Glu Asp Lys Lys Leu 720

721 Ala Ile Thr Thr Leu Thr Asn Thr Leu Asn Gln Val Gln Lys Thr Gln 736

737 Gln Gly Val Ala Val Ser Leu Leu Ala Gln Thr Leu Asn Val Ser Gln 752

753 Ser Leu Pro Ala Leu Leu Leu Arg Trp Ser Gly Gln Thr Thr Tyr Gln 768

769 Trp Leu Ser Ala Thr Trp Ala Leu Lys Asp Ala Val Lys Thr Ala Ala 784

785 Asp Ile Pro Ala Asp Tyr Leu Arg Gln Leu Arg Glu Val Val Arg Arg 800

801 Ser Leu Leu Thr Gln Gln Phe Thr Leu Ser Pro Ala Met Val Gln Thr 816

817 Leu Leu Asp Tyr Pro Ala Tyr Phe Gly Ala Ser Ala Glu Thr Val Thr 832

833 Asp Ile Ser Leu Trp Met Leu Tyr Thr Leu Ser Cys Tyr Ser Asp Leu 848

849 Leu Leu Gln Met Gly Glu Ala Gly Gly Thr Glu Asp Asp Val Leu Ala 864

865 Tyr Leu Arg Thr Ala Asn Ala Thr Thr Pro Leu Ser Gln Ser Asp Ala 880

881 Ala Gln Thr Leu Ala Thr Leu Leu Gly Trp Glu Val Asn Glu Leu Gln 896

897 Ala Ala Trp Ser Val Leu Gly Gly Ile Ala Lys Thr Thr Pro Gln Leu 912

913 Asp Ala Leu Leu Arg Leu Gln Gln Ala Gln Asn Gln Thr Gly Leu Gly 928

929 Val Thr Gln Gln Gln Gln Gly Tyr Leu Leu Ser Arg Asp Ser Asp Tyr 944

945 Thr Leu Trp Gln Ser Thr Gly Gln Ala Leu Val Ala Gly Val Ser His 960

961 Val Lys Gly Ser Asn 965

(2) INFORMATION FOR SEQ ID NO:58

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4698 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58 (*tccB*)

1 ATG TTA TCG ACA ATG GAA AAA CAA CTG AAT GAA TCC CAG CGT GAT GCG 48
1 Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu Ser Gln Arg Asp Ala 16

49 TTG GTG ACT GGC TAT ATG AAT TTT GTG GCG CCG ACG TTG AAA GGC GTC 96
17 Leu Val Thr Gly Tyr Met Asn Phe Val Ala Pro Thr Leu Lys Gly Val 32

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97  AGT GGT CAG CCG GTG ACG GTG GAA GAT TTA TAC GAA TAT TTG CTG ATT  144
33  Ser Gly Gln Pro Val Thr Val Glu Asp Leu Tyr Glu Tyr Leu Leu Ile   48

145 GAC CCG GAA GTG GCT GAT GAG GTT GAG ACG AGT CGG GTA GCA CAA GCG  192
49  Asp Pro Glu Val Ala Asp Glu Val Glu Thr Ser Arg Val Ala Gln Ala   64

193 ATT GCC AGC ATA CAG CAA TAT ATG ACT CGT CTG GTC AAC GGC TCT GAA  240
65  Ile Ala Ser Ile Gln Gln Tyr Met Thr Arg Leu Val Asn Gly Ser Glu   80

241 CCG GGG CGT CAG GCG ATG GAG CCT TCT ACA GCT AAC GAA TGG CGT GAT  288
81  Pro Gly Arg Gln Ala Met Glu Pro Ser Thr Ala Asn Glu Trp Arg Asp   96

289 AAT GAT AAC CAA TAT GCT ATC TGG GCT GCG GGG GCT GAG GTT CGA AAT  336
97  Asn Asp Asn Gln Tyr Ala Ile Trp Ala Ala Gly Ala Glu Val Arg Asn  112

337 TAC GCT GAA AAC TAT ATT TCA CCC ATC ACC CGG CAG GAA AAA AGC CAT  384
113 Tyr Ala Glu Asn Tyr Ile Ser Pro Ile Thr Arg Gln Glu Lys Ser His  128

385 TAT TTC TCG GAG CTG GAG ACG ACT TTA AAT CAG AAT CGA CTC GAT CCG  432
129 Tyr Phe Ser Glu Leu Glu Thr Thr Leu Asn Gln Asn Arg Leu Asp Pro  144

433 GAT CGT GTG CAG GAT GCT GTT TTG GCG TAT CTC AAT GAG TTT GAG GCA  480
145 Asp Arg Val Gln Asp Ala Val Leu Ala Tyr Leu Asn Glu Phe Glu Ala  160

481 GTG AGT AAT CTA TAT GTG CTC AGT GGT TAT ATT AAT CAG GAT AAA TTT  528
161 Val Ser Asn Leu Tyr Val Leu Ser Gly Tyr Ile Asn Gln Asp Lys Phe  176

529 GAC CAA GCT ATC TAC TAC TTT ATT GGT CGC ACT ACC ACT AAA CCG TAT  576
177 Asp Gln Ala Ile Tyr Tyr Phe Ile Gly Arg Thr Thr Thr Lys Pro Tyr  192

577 CGC TAC TAC TGG CGT CAG ATG GAT TTG AGT AAG AAC CGT CAA GAT CCG  624
193 Arg Tyr Tyr Trp Arg Gln Met Asp Leu Ser Lys Asn Arg Gln Asp Pro  208

625 GCA GGG AAT CCG GTG ACG CCA AAT TGC TGG AAT GAT TGG CAG GAA ATC  672
209 Ala Gly Asn Pro Val Thr Pro Asn Cys Trp Asn Asp Trp Gln Glu Ile  224

673 ACT TTG CCG CTG TCT GGT GAT ACG GTG CTG GAG CAT ACA GTT CGC CCG  720
225 Thr Leu Pro Leu Ser Gly Asp Thr Val Leu Glu His Thr Val Arg Pro  240

721 GTA TTT TAT AAT GAT CGA CTA TAT GTG GCT TGG GTT GAG CGT GAC CCG  768
241 Val Phe Tyr Asn Asp Arg Leu Tyr Val Ala Trp Val Glu Arg Asp Pro  256

769 GCA GTA CAG AAG GAT GCT GAC GGT AAA AAC ATC GGT AAA ACC CAT GCC  816
257 Ala Val Gln Lys Asp Ala Asp Gly Lys Asn Ile Gly Lys Thr His Ala  272

817 TAC AAC ATA AAG TTT GGT TAT AAA CGT TAT GAT GAT ACT TGG ACA GCG  864
273 Tyr Asn Ile Lys Phe Gly Tyr Lys Arg Tyr Asp Asp Thr Trp Thr Ala  288

865 CCG AAT ACG ACC ACG TTA ATG ACA CAA CAA GCA GGG GAA AGT TCA GAA  912
289 Pro Asn Thr Thr Thr Leu Met Thr Gln Gln Ala Gly Glu Ser Ser Glu  304
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 913  ACA CAG CGA TCC AGC CTG CTG ATT GAT GAA TCT AGC ACC ACA TTG CGC   960
 305  Thr Gln Arg Ser Ser Leu Leu Ile Asp Glu Ser Ser Thr Thr Leu Arg   320

 961  CAA GTT AAT CTG TTG GCT ACC ACC GAT TTT AGT ATC GAT CCG ACG GAG  1008
 321  Gln Val Asn Leu Leu Ala Thr Thr Asp Phe Ser Ile Asp Pro Thr Glu   336

1009  GAA ACG GAC AGT AAC CCG TAT GGC CGC CTA ATG TTG GGG GTG TTT GTC  1056
 337  Glu Thr Asp Ser Asn Pro Tyr Gly Arg Leu Met Leu Gly Val Phe Val   352

1057  CGT CAA TTT GAA GGT GAT GGG GCC AAT AGA AAA AAT AAA CCC GTT GTT  1104
 353  Arg Gln Phe Glu Gly Asp Gly Ala Asn Arg Lys Asn Lys Pro Val Val   368

1105  TAT GGT TAT CTC TAT TGT GAC TCA GCT TTC AAT CGT CAT GTT CTC AGG  1152
 369  Tyr Gly Tyr Leu Tyr Cys Asp Ser Ala Phe Asn Arg His Val Leu Arg   384

1153  CCG TTA AGT AAG AAC TTT TTG TTC AGT ACT TAC CGT GAT GAA ACG GAT  1200
 385  Pro Leu Ser Lys Asn Phe Leu Phe Ser Thr Tyr Arg Asp Glu Thr Asp   400

1201  GGT CAA AAC AGC TTG CAA TTT GCG GTA TAC GAT AAA AAG TAT GTA ATT  1248
 401  Gly Gln Asn Ser Leu Gln Phe Ala Val Tyr Asp Lys Lys Tyr Val Ile   416

1249  ACT AAG GTT GTT ACA GGT GCA ACG GAA GAT CCC GAA AAT ACA GGA TGG  1296
 417  Thr Lys Val Val Thr Gly Ala Thr Glu Asp Pro Glu Asn Thr Gly Trp   432

1297  GTA AGT AAA GTT GAT GAC TTG AAA CAA GGC ACT ACT GGG GCC TAT GTG  1344
 433  Val Ser Lys Val Asp Asp Leu Lys Gln Gly Thr Thr Gly Ala Tyr Val   448

1345  TAT ATC GAT CAA GAT GGC CTG ACG CTT CAT ATA CAA ACC ACA ACT AAT  1392
 449  Tyr Ile Asp Gln Asp Gly Leu Thr Leu His Ile Gln Thr Thr Thr Asn   464

1393  GGG GAT TTT ATT AAC CGT CAT ACG TTT GGA TAT AAC GAT CTT GTA TAT  1440
 465  Gly Asp Phe Ile Asn Arg His Thr Phe Gly Tyr Asn Asp Leu Val Tyr   480

1441  GAT TCT AAG TCT GGT TAT GGT TTC ACG TGG TCA GGA AAT GAA GGT TTT  1488
 481  Asp Ser Lys Ser Gly Tyr Gly Phe Thr Trp Ser Gly Asn Glu Gly Phe   496

1489  TAT CTG GAT TAC CAT GAT GGA AAT TAT TAC ACC TTT CAT AAT GCA ATA  1536
 497  Tyr Leu Asp Tyr His Asp Gly Asn Tyr Tyr Thr Phe His Asn Ala Ile   512

1537  ATC AAC TAC TAT CCG TCT GGA TAT GGT GGT GGA TCT GTT CCT AAT GGA  1584
 513  Ile Asn Tyr Tyr Pro Ser Gly Tyr Gly Gly Gly Ser Val Pro Asn Gly   528

1585  ACG TGG GCG TTA GAG CAA AGG ATT AAT GAG GGA TGG GCT ATT GCT CCC  1632
 529  Thr Trp Ala Leu Glu Gln Arg Ile Asn Glu Gly Trp Ala Ile Ala Pro   544

1633  CTG CTT GAT ACT CTC CAT ACT GTT ACT GTG AAG GGC AGT TAT ATC GCT  1680
 545  Leu Leu Asp Thr Leu His Thr Val Thr Val Lys Gly Ser Tyr Ile Ala   560
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1681  TGG GAA GGG GAA ACA CCT ACC GGT TAT AAT CTG TAT ATT CCA GAT GGT  1728
 561  Trp Glu Gly Glu Thr Pro Thr Gly Tyr Asn Leu Tyr Ile Pro Asp Gly   576

1729  ACC GTG TTG CTA GAT TGG TTT GAT AAA ATA AAT TTT GCT ATT GGT CTT  1776
 577  Thr Val Leu Leu Asp Trp Phe Asp Lys Ile Asn Phe Ala Ile Gly Leu   592

1777  AAT AAG CTT GAG TCT GTA TTT ACG TCG CCA GAT TGG CCA ACA CTA ACC  1824
 593  Asn Lys Leu Glu Ser Val Phe Thr Ser Pro Asp Trp Pro Thr Leu Thr   608

1825  ACT ATC AAA AAT TTC AGT AAA ATC GCC GAT AAC CGC AAA TTC TAT CAG  1872
 609  Thr Ile Lys Asn Phe Ser Lys Ile Ala Asp Asn Arg Lys Phe Tyr Gln   624

1873  GAA ATC AAT GCT GAG ACG GCG GAT GGA CGC AAC CTG TTT AAA CGT TAC  1920
 625  Glu Ile Asn Ala Glu Thr Ala Asp Gly Arg Asn Leu Phe Lys Arg Tyr   640

1921  AGT ACT CAA ACT TTC GGA CTT ACC AGC GGT GCG ACT TAT TCT ACA ACT  1968
 641  Ser Thr Gln Thr Phe Gly Leu Thr Ser Gly Ala Thr Tyr Ser Thr Thr   656

1969  TAT ACT TTG TCT GAG GCG GAT TTC TCC ACT GAT CCG GAC AAA AAC TAC  2016
 657  Tyr Thr Leu Ser Glu Ala Asp Phe Ser Thr Asp Pro Asp Lys Asn Tyr   672

2017  CTA CAG GTT TGT TTG AAT GTC GTG TGG GAT CAT TAT GAC CGC CCG TCA  2064
 673  Leu Gln Val Cys Leu Asn Val Val Trp Asp His Tyr Asp Arg Pro Ser   688

2065  GGG AAA AAA GGG GCT TAT TCT TGG GTC AGT AAG TGG TTT AAC GTC TAT  2112
 689  Gly Lys Lys Gly Ala Tyr Ser Trp Val Ser Lys Trp Phe Asn Val Tyr   704

2113  GTT GCG TTG CAA GAT AGC AAA GCT CCG GAT GCC ATT CCT CGA TTA GTT  2160
 705  Val Ala Leu Gln Asp Ser Lys Ala Pro Asp Ala Ile Pro Arg Leu Val   720

2161  TCC CGT TAC GAT AGT AAA CGT GGT CTG GTG CAA TAT CTG GAC TTC TGG  2208
 721  Ser Arg Tyr Asp Ser Lys Arg Gly Leu Val Gln Tyr Leu Asp Phe Trp   736

2209  ACC TCA TCA TTA CCC GCG AAA ACC CGT CTT AAC ACC ACC TTT GTG CGT  2256
 737  Thr Ser Ser Leu Pro Ala Lys Thr Arg Leu Asn Thr Thr Phe Val Arg   752

2257  ACT TTG ATT GAG AAG GCT AAT CTG GGG CTG GAT AGT TTG CTG GAT TAC  2304
 753  Thr Leu Ile Glu Lys Ala Asn Leu Gly Leu Asp Ser Leu Leu Asp Tyr   768

2305  ACC TTG CAG GCA GAT CCT TCT CTG GAA GCA GAT TTA GTG ACT GAC GGC  2352
 769  Thr Leu Gln Ala Asp Pro Ser Leu Glu Ala Asp Leu Val Thr Asp Gly   784

2353  AAA AGC GAA CCA ATG GAC TTT AAT GGT TCA AAC GGT CTC TAT TTC TGG  2400
 785  Lys Ser Glu Pro Met Asp Phe Asn Gly Ser Asn Gly Leu Tyr Phe Trp   800

2401  GAA TTG TTC TTT CAC CTG CCG TTT TTG GTT GCT ACA CGC TTT GCC AAC  2448
 801  Glu Leu Phe Phe His Leu Pro Phe Leu Val Ala Thr Arg Phe Ala Asn   816

2449  GAA CAG CAA TTT TCG CCG GCA CAA AAG AGT TTG CAT TAC ATC TTT GAC  2496
 817  Glu Gln Gln Phe Ser Pro Ala Gln Lys Ser Leu His Tyr Ile Phe Asp   832
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2497  CCG GCG ATG AAA AAC AAG CCA CAC AAT GCC CCG GCT TAT TGG AAT GTA  2544
 833  Pro Ala Met Lys Asn Lys Pro His Asn Ala Pro Ala Tyr Trp Asn Val   848

2545  CGT CCG TTG GTT GAA GGA AAC AGC GAT TTG TCA CGT CAT TTG GAC GAT  2592
 849  Arg Pro Leu Val Glu Gly Asn Ser Asp Leu Ser Arg His Leu Asp Asp   864

2593  TCT ATA GAC CCA GAT ACT CAA GCT TAT GCT CAT CCG GTG ATA TAC CAG  2640
 865  Ser Ile Asp Pro Asp Thr Gln Ala Tyr Ala His Pro Val Ile Tyr Gln   880

2641  AAA GCG GTG TTT ATT GCC TAT GTC AGT AAC CTG ATT GCT CAG GGA GAT  2688
 881  Lys Ala Val Phe Ile Ala Tyr Val Ser Asn Leu Ile Ala Gln Gly Asp   896

2689  ATG TGG TAT CGC CAA TTG ACT CGT GAC GGT CTG ACT CAG GCC CGT GTC  2736
 897  Met Trp Tyr Arg Gln Leu Thr Arg Asp Gly Leu Thr Gln Ala Arg Val   912

2737  TAT TAC AAT CTG GCC GCT GAA TTG CTA GGG CCT CGT CCG GAT GTA TCG  2784
 913  Tyr Tyr Asn Leu Ala Ala Glu Leu Leu Gly Pro Arg Pro Asp Val Ser   928

2785  CTG AGT AGC ATT TGG ACG CCG CAA ACC CTG GAT ACC TTA GCA GCC GGG  2832
 929  Leu Ser Ser Ile Trp Thr Pro Gln Thr Leu Asp Thr Leu Ala Ala Gly   944

2833  CAA AAA GCG GTT TTA CGT GAT TTT GAG CAC CAG TTG GCT AAT AGT GAT  2880
 945  Gln Lys Ala Val Leu Arg Asp Phe Glu His Gln Leu Ala Asn Ser Asp   960

2881  ACC GCT TTA CCC GCA TTG CCG GGC CGC AAT GTC AGC TAC TTG AAA CTG  2928
 961  Thr Ala Leu Pro Ala Leu Pro Gly Arg Asn Val Ser Tyr Leu Lys Leu   976

2929  GCA GAT AAT GGC TAC TTT AAT GAA CCG CTC AAT GTT CTG ATG TTG TCT  2976
 977  Ala Asp Asn Gly Tyr Phe Asn Glu Pro Leu Asn Val Leu Met Leu Ser   992

2977  CAC TGG GAT ACG TTG GAT GCA CGG TTA TAC AAT CTG CGT CAT AAC CTG  3024
 993  His Trp Asp Thr Leu Asp Ala Arg Leu Tyr Asn Leu Arg His Asn Leu  1008

3025  ACC GTT GAT GGC AAG CCG CTT TCG CTG CCG CTG TAT GCT GCG CCT GTT  3072
1009  Thr Val Asp Gly Lys Pro Leu Ser Leu Pro Leu Tyr Ala Ala Pro Val  1024

3073  GAT CCG GTA GCG TTG TTG GCT CAG CGT GCT CAG TCC GGC ACG TTG ACG  3120
1025  Asp Pro Val Ala Leu Leu Ala Gln Arg Ala Gln Ser Gly Thr Leu Thr  1040

3121  AAT GGC GTC AGT GGC GCC ATG TTG ACG GTG CCG CCA TAC CGT TTC AGC  3168
1041  Asn Gly Val Ser Gly Ala Met Leu Thr Val Pro Pro Tyr Arg Phe Ser  1056

3169  GCT ATG TTG CCG CGA GCT TAC AGC GCC GTG GGT ACG TTG ACC AGT TTT  3216
1057  Ala Met Leu Pro Arg Ala Tyr Ser Ala Val Gly Thr Leu Thr Ser Phe  1072

3217  GGT CAG AAC CTG CTT AGT TTG TTG GAA CGT AGC GAA CGA GCC TGT CAA  3264
1073  Gly Gln Asn Leu Leu Ser Leu Leu Glu Arg Ser Glu Arg Ala Cys Gln  1088
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3265  GAA GAG TTG GCG CAA CAG CAA CTG TTG GAT ATG TCC AGC TAT GCG ATC   3312
1089  Glu Glu Leu Ala Gln Gln Gln Leu Leu Asp Met Ser Ser Tyr Ala Ile   1104

3313  ACG TTG CAA CAA CAG GCG CTG GAT GGA TTG GCG GCA GAT CGT CTG GCG   3360
1105  Thr Leu Gln Gln Gln Ala Leu Asp Gly Leu Ala Ala Asp Arg Leu Ala   1120

3361  CTG CTA GCT AGT CAG GCT ACG GCA CAA CAG CGT CAT GAC CAT TAT TAC   3408
1121  Leu Leu Ala Ser Gln Ala Thr Ala Gln Gln Arg His Asp His Tyr Tyr   1136

3409  ACT CTG TAT CAG AAC AAC ATC TCC AGT GCG GAA CAA CTG GTG ATG GAC   3456
1137  Thr Leu Tyr Gln Asn Asn Ile Ser Ser Ala Glu Gln Leu Val Met Asp   1152

3457  ACC CAA ACG TCA GCA CAA TCC CTG ATT TCT TCT TCC ACT GGT GTA CAA   3504
1153  Thr Gln Thr Ser Ala Gln Ser Leu Ile Ser Ser Ser Thr Gly Val Gln   1168

3505  ACT GCC AGT GGG GCA CTG AAA GTG ATC CCG AAT ATC TTT GGT TTG GCT   3552
1169  Thr Ala Ser Gly Ala Leu Lys Val Ile Pro Asn Ile Phe Gly Leu Ala   1184

3553  GAT GGC GGC TCG CGC TAT GAA GGA GTA ACG GAA GCG ATT GCC ATC GGG   3600
1185  Asp Gly Gly Ser Arg Tyr Glu Gly Val Thr Glu Ala Ile Ala Ile Gly   1200

3601  TTA ATG GCT GCC GGA CAA GCC ACC AGC GTG GTG GCC GAG CGT CTG GCA   3648
1201  Leu Met Ala Ala Gly Gln Ala Thr Ser Val Val Ala Glu Arg Leu Ala   1216

3649  ACC ACG GAG AAT TAC CGC CGC CGC CGT GAA GAG TGG CAA ATC CAA TAC   3696
1217  Thr Thr Glu Asn Tyr Arg Arg Arg Arg Glu Glu Trp Gln Ile Gln Tyr   1232

3697  CAG CAG GCA CAG TCT GAG GTC GAC GCA TTA CAG AAA CAG TTG GAT GCG   3744
1233  Gln Gln Ala Gln Ser Glu Val Asp Ala Leu Gln Lys Gln Leu Asp Ala   1248

3745  CTG GCA GTG CGC GAG AAA GCA GCT CAA ACT TCC CTG CAA CAG GCG AAG   3792
1249  Leu Ala Val Arg Glu Lys Ala Ala Gln Thr Ser Leu Gln Gln Ala Lys   1264

3793  GCA CAG CAG GTA CAA ATT CGG ACC ATG CTG ACT TAC TTA ACT ACT CGT   3840
1265  Ala Gln Gln Val Gln Ile Arg Thr Met Leu Thr Tyr Leu Thr Thr Arg   1280

3841  TTC ACC CAG GCG ACT CTG TAC CAG TGG CTG AGT GGT CAA TTA TCC GCG   3888
1281  Phe Thr Gln Ala Thr Leu Tyr Gln Trp Leu Ser Gly Gln Leu Ser Ala   1296

3889  TTG TAT TAT CAA GCG TAT GAT GCC GTG GTT GCT CTC TGC CTC TCC GCC   3936
1297  Leu Tyr Tyr Gln Ala Tyr Asp Ala Val Val Ala Leu Cys Leu Ser Ala   1312

3937  CAA GCT TGC TGG CAG TAT GAA TTG GGT GAT TAC GCT ACC ACT TTT ATC   3984
1313  Gln Ala Cys Trp Gln Tyr Glu Leu Gly Asp Tyr Ala Thr Thr Phe Ile   1328

3985  CAG ACC GGT ACC TGG AAC GAC CAT TAC CGT GGT TTG CAA GTG GGG GAG   4032
1329  Gln Thr Gly Thr Trp Asn Asp His Tyr Arg Gly Leu Gln Val Gly Glu   1344

4033  ACA CTG CAA CTC AAT TTG CAT CAG ATG GAA GCG GCC TAT TTA GTT CGT   4080
1345  Thr Leu Gln Leu Asn Leu His Gln Met Glu Ala Ala Tyr Leu Val Arg   1360
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4081  CAC GAA CGC CGT CTT AAT GTG ATC CGT ACT GTG TCG CTC AAA AGC CTA  4128
1361  His Glu Arg Arg Leu Asn Val Ile Arg Thr Val Ser Leu Lys Ser Leu  1376

4129  TTG GGT GAT GAT GGT TTT GGT AAG TTA AAA ACC GAA GGC AAA GTC GAC  4176
1377  Leu Gly Asp Asp Gly Phe Gly Lys Leu Lys Thr Glu Gly Lys Val Asp  1392

4177  TTT CCA TTA AGC GAA AAG CTG TTT GAC AAC GAC TAT CCG GGG CAC TAT  4224
1393  Phe Pro Leu Ser Glu Lys Leu Phe Asp Asn Asp Tyr Pro Gly His Tyr  1408

4225  TTG CGC CAG ATT AAA ACT GTG TCA GTG ACG TTG CCG ACG TTA GTC GGG  4272
1409  Leu Arg Gln Ile Lys Thr Val Ser Val Thr Leu Pro Thr Leu Val Gly  1424

4273  CCG TAT CAA AAC GTG AAG GCA ACG CTC ACT CAG ACC AGC AGC AGT ATA  4320
1425  Pro Tyr Gln Asn Val Lys Ala Thr Leu Thr Gln Thr Ser Ser Ser Ile  1440

4321  TTG TTA GCA GCA GAT ATC AAT GGT GTT AAA CGT CTC AAT GAT CCG ACA  4368
1441  Leu Leu Ala Ala Asp Ile Asn Gly Val Lys Arg Leu Asn Asp Pro Thr  1456

4369  GGT AAA GAG GGT GAT GCG ACG CAT ATT GTC ACC AAT CTG CGT GCC AGC  4416
1457  Gly Lys Glu Gly Asp Ala Thr His Ile Val Thr Asn Leu Arg Ala Ser  1472

4417  CAG CAG GTG GCG CTC TCT TCT GGC ATT AAT GAT GCC GGT AGC TTT GAG  4464
1473  Gln Gln Val Ala Leu Ser Ser Gly Ile Asn Asp Ala Gly Ser Phe Glu  1488

4465  TTG CGT TTG GAA GAT GAG CGC TAT CTA TCA TTT GAG GGG ACT GGA GCT  4512
1489  Leu Arg Leu Glu Asp Glu Arg Tyr Leu Ser Phe Glu Gly Thr Gly Ala  1504

4513  GTT TCC AAA TGG ACT CTT AAC TTC CCG CGT TCT GTG GAT GAG CAT ATT  4560
1505  Val Ser Lys Trp Thr Leu Asn Phe Pro Arg Ser Val Asp Glu His Ile  1520

4561  GAC GAT AAG ACA TTG AAA GCG GAT GAG ATG CAG GCC GCA CTG TTG GCG  4608
1521  Asp Asp Lys Thr Leu Lys Ala Asp Glu Met Gln Ala Ala Leu Leu Ala  1536

4609  AAT ATG GAT GAT GTG CTG GTG CAG GTG CAT TAT ACC GCC TGC GAC GGC  4656
1537  Asn Met Asp Asp Val Leu Val Gln Val His Tyr Thr Ala Cys Asp Gly  1552

4657  GGC GCC AGT TTC GCA AAC CAG GTC AAG AAA ACA CTC TCT TAA   4698
1553  Gly Ala Ser Phe Ala Asn Gln Val Lys Lys Thr Leu Ser End   1566
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(2) INFORMATION FOR SEQ ID NO:59

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1665 amino acids

(B) TYPE: amino acid

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59 (TccB peptide)

| Features | From | To | Description |
|---|---|---|---|

i 11 SEQ ID NO:7

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Met | Leu | Ser | Thr | Met | Glu | Lys | Gln | Leu | Asn | Glu | Ser | Gln | Arg | Asp | Ala | 16 |
| 17 | Leu | Val | Thr | Gly | Tyr | Met | Asn | Phe | Val | Ala | Pro | Thr | Leu | Lys | Gly | Val | 32 |
| 33 | Ser | Gly | Gln | Pro | Val | Thr | Val | Glu | Asp | Leu | Tyr | Glu | Tyr | Leu | Leu | Ile | 48 |
| 49 | Asp | Pro | Glu | Val | Ala | Asp | Glu | Val | Glu | Thr | Ser | Arg | Val | Ala | Gln | Ala | 64 |
| 65 | Ile | Ala | Ser | Ile | Gln | Gln | Tyr | Met | Thr | Arg | Leu | Val | Asn | Gly | Ser | Glu | 80 |
| 81 | Pro | Gly | Arg | Gln | Ala | Met | Glu | Pro | Ser | Thr | Ala | Asn | Glu | Trp | Arg | Asp | 96 |
| 97 | Asn | Asp | Asn | Gln | Tyr | Ala | Ile | Trp | Ala | Ala | Gly | Ala | Glu | Val | Arg | Asn | 112 |
| 113 | Tyr | Ala | Glu | Asn | Tyr | Ile | Ser | Pro | Ile | Thr | Arg | Gln | Glu | Lys | Ser | His | 128 |
| 129 | Tyr | Phe | Ser | Glu | Leu | Glu | Thr | Thr | Leu | Asn | Gln | Asn | Arg | Leu | Asp | Pro | 144 |
| 145 | Asp | Arg | Val | Gln | Asp | Ala | Val | Leu | Ala | Tyr | Leu | Asn | Glu | Phe | Glu | Ala | 160 |
| 161 | Val | Ser | Asn | Leu | Tyr | Val | Leu | Ser | Gly | Tyr | Ile | Asn | Gln | Asp | Lys | Phe | 176 |
| 177 | Asp | Gln | Ala | Ile | Tyr | Tyr | Phe | Ile | Gly | Arg | Thr | Thr | Thr | Lys | Pro | Tyr | 192 |
| 193 | Arg | Tyr | Tyr | Trp | Arg | Gln | Met | Asp | Leu | Ser | Lys | Asn | Arg | Gln | Asp | Pro | 208 |
| 209 | Ala | Gly | Asn | Pro | Val | Thr | Pro | Asn | Cys | Trp | Asn | Asp | Trp | Gln | Glu | Ile | 224 |
| 225 | Thr | Leu | Pro | Leu | Ser | Gly | Asp | Thr | Val | Leu | Glu | His | Thr | Val | Arg | Pro | 240 |
| 241 | Val | Phe | Tyr | Asn | Asp | Arg | Leu | Tyr | Val | Ala | Trp | Val | Glu | Arg | Asp | Pro | 256 |
| 257 | Ala | Val | Gln | Lys | Asp | Ala | Asp | Gly | Lys | Asn | Ile | Gly | Lys | Thr | His | Ala | 272 |
| 273 | Tyr | Asn | Ile | Lys | Phe | Gly | Tyr | Lys | Arg | Tyr | Asp | Asp | Thr | Trp | Thr | Ala | 288 |
| 289 | Pro | Asn | Thr | Thr | Thr | Leu | Met | Thr | Gln | Gln | Ala | Gly | Glu | Ser | Ser | Glu | 304 |
| 305 | Thr | Gln | Arg | Ser | Ser | Leu | Leu | Ile | Asp | Glu | Ser | Ser | Thr | Thr | Leu | Arg | 320 |
| 321 | Gln | Val | Asn | Leu | Leu | Ala | Thr | Thr | Asp | Phe | Ser | Ile | Asp | Pro | Thr | Glu | 336 |
| 337 | Glu | Thr | Asp | Ser | Asn | Pro | Tyr | Gly | Arg | Leu | Met | Leu | Gly | Val | Phe | Val | 352 |
| 353 | Arg | Gln | Phe | Glu | Gly | Asp | Gly | Ala | Asn | Arg | Lys | Asn | Lys | Pro | Val | Val | 368 |
| 369 | Tyr | Gly | Tyr | Leu | Tyr | Cys | Asp | Ser | Ala | Phe | Asn | Arg | His | Val | Leu | Arg | 384 |
| 385 | Pro | Leu | Ser | Lys | Asn | Phe | Leu | Phe | Ser | Thr | Tyr | Arg | Asp | Glu | Thr | Asp | 400 |
| 401 | Gly | Gln | Asn | Ser | Leu | Gln | Phe | Ala | Val | Tyr | Asp | Lys | Lys | Tyr | Val | Ile | 416 |
| 417 | Thr | Lys | Val | Val | Thr | Gly | Ala | Thr | Glu | Asp | Pro | Glu | Asn | Thr | Gly | Trp | 432 |
| 433 | Val | Ser | Lys | Val | Asp | Asp | Leu | Lys | Gln | Gly | Thr | Thr | Gly | Ala | Tyr | Val | 448 |
| 449 | Tyr | Ile | Asp | Gln | Asp | Gly | Leu | Thr | Leu | His | Ile | Gln | Thr | Thr | Thr | Asn | 464 |
| 465 | Gly | Asp | Phe | Ile | Asn | Arg | His | Thr | Phe | Gly | Tyr | Asn | Asp | Leu | Val | Tyr | 480 |
| 481 | Asp | Ser | Lys | Ser | Gly | Tyr | Gly | Phe | Thr | Trp | Ser | Gly | Asn | Glu | Gly | Phe | 496 |
| 497 | Tyr | Leu | Asp | Tyr | His | Asp | Gly | Asn | Tyr | Tyr | Thr | Phe | His | Asn | Ala | Ile | 512 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 513 | Ile | Asn | Tyr | Tyr | Pro | Ser | Gly | Tyr | Gly | Gly | Gly | Ser | Val | Pro | Asn | Gly | 528 |
| 529 | Thr | Trp | Ala | Leu | Glu | Gln | Arg | Ile | Asn | Glu | Gly | Trp | Ala | Ile | Ala | Pro | 544 |
| 545 | Leu | Leu | Asp | Thr | Leu | His | Thr | Val | Thr | Val | Lys | Gly | Ser | Tyr | Ile | Ala | 560 |
| 561 | Trp | Glu | Gly | Glu | Thr | Pro | Thr | Gly | Tyr | Asn | Leu | Tyr | Ile | Pro | Asp | Gly | 576 |
| 577 | Thr | Val | Leu | Leu | Asp | Trp | Phe | Asp | Lys | Ile | Asn | Phe | Ala | Ile | Gly | Leu | 592 |
| 593 | Asn | Lys | Leu | Glu | Ser | Val | Phe | Thr | Ser | Pro | Asp | Trp | Pro | Thr | Leu | Thr | 608 |
| 609 | Thr | Ile | Lys | Asn | Phe | Ser | Lys | Ile | Ala | Asp | Asn | Arg | Lys | Phe | Tyr | Gln | 624 |
| 625 | Glu | Ile | Asn | Ala | Glu | Thr | Ala | Asp | Gly | Arg | Asn | Leu | Phe | Lys | Arg | Tyr | 640 |
| 641 | Ser | Thr | Gln | Thr | Phe | Gly | Leu | Thr | Ser | Gly | Ala | Thr | Tyr | Ser | Thr | Thr | 656 |
| 657 | Tyr | Thr | Leu | Ser | Glu | Ala | Asp | Phe | Ser | Thr | Asp | Pro | Asp | Lys | Asn | Tyr | 672 |
| 673 | Leu | Gln | Val | Cys | Leu | Asn | Val | Val | Trp | Asp | His | Tyr | Asp | Arg | Pro | Ser | 688 |
| 689 | Gly | Lys | Lys | Gly | Ala | Tyr | Ser | Trp | Val | Ser | Lys | Trp | Phe | Asn | Val | Tyr | 704 |
| 705 | Val | Ala | Leu | Gln | Asp | Ser | Lys | Ala | Pro | Asp | Ala | Ile | Pro | Arg | Leu | Val | 720 |
| 721 | Ser | Arg | Tyr | Asp | Ser | Lys | Arg | Gly | Leu | Val | Gln | Tyr | Leu | Asp | Phe | Trp | 736 |
| 737 | Thr | Ser | Ser | Leu | Pro | Ala | Lys | Thr | Arg | Leu | Asn | Thr | Thr | Phe | Val | Arg | 752 |
| 753 | Thr | Leu | Ile | Glu | Lys | Ala | Asn | Leu | Gly | Leu | Asp | Ser | Leu | Leu | Asp | Tyr | 768 |
| 769 | Thr | Leu | Gln | Ala | Asp | Pro | Ser | Leu | Glu | Ala | Asp | Leu | Val | Thr | Asp | Gly | 784 |
| 785 | Lys | Ser | Glu | Pro | Met | Asp | Phe | Asn | Gly | Ser | Asn | Gly | Leu | Tyr | Phe | Trp | 800 |
| 801 | Glu | Leu | Phe | Phe | His | Leu | Pro | Phe | Leu | Val | Ala | Thr | Arg | Phe | Ala | Asn | 816 |
| 817 | Glu | Gln | Gln | Phe | Ser | Pro | Ala | Gln | Lys | Ser | Leu | His | Tyr | Ile | Phe | Asp | 832 |
| 833 | Pro | Ala | Met | Lys | Asn | Lys | Pro | His | Asn | Ala | Pro | Ala | Tyr | Trp | Asn | Val | 848 |
| 849 | Arg | Pro | Leu | Val | Glu | Gly | Asn | Ser | Asp | Leu | Ser | Arg | His | Leu | Asp | Asp | 864 |
| 865 | Ser | Ile | Asp | Pro | Asp | Thr | Gln | Ala | Tyr | Ala | His | Pro | Val | Ile | Tyr | Gln | 880 |
| 881 | Lys | Ala | Val | Phe | Ile | Ala | Tyr | Val | Ser | Asn | Leu | Ile | Ala | Gln | Gly | Asp | 896 |
| 897 | Met | Trp | Tyr | Arg | Gln | Leu | Thr | Arg | Asp | Gly | Leu | Thr | Gln | Ala | Arg | Val | 912 |
| 913 | Tyr | Tyr | Asn | Leu | Ala | Ala | Glu | Leu | Leu | Gly | Pro | Arg | Pro | Asp | Val | Ser | 928 |
| 929 | Leu | Ser | Ser | Ile | Trp | Thr | Pro | Gln | Thr | Leu | Asp | Thr | Leu | Ala | Ala | Gly | 944 |
| 945 | Gln | Lys | Ala | Val | Leu | Arg | Asp | Phe | Glu | His | Gln | Leu | Ala | Asn | Ser | Asp | 960 |
| 961 | Thr | Ala | Leu | Pro | Ala | Leu | Pro | Gly | Arg | Asn | Val | Ser | Tyr | Leu | Lys | Leu | 976 |
| 977 | Ala | Asp | Asn | Gly | Tyr | Phe | Asn | Glu | Pro | Leu | Asn | Val | Leu | Met | Leu | Ser | 992 |
| 993 | His | Trp | Asp | Thr | Leu | Asp | Ala | Arg | Leu | Tyr | Asn | Leu | Arg | His | Asn | Leu | 1008 |
| 1009 | Thr | Val | Asp | Gly | Lys | Pro | Leu | Ser | Leu | Pro | Leu | Tyr | Ala | Ala | Pro | Val | 1024 |
| 1025 | Asp | Pro | Val | Ala | Leu | Leu | Ala | Gln | Arg | Ala | Gln | Ser | Gly | Thr | Leu | Thr | 1040 |

1041 Asn Gly Val Ser Gly Ala Met Leu Thr Val Pro Pro Tyr Arg Phe Ser 1056
1057 Ala Met Leu Pro Arg Ala Tyr Ser Ala Val Gly Thr Leu Thr Ser Phe 1072
1073 Gly Gln Asn Leu Leu Ser Leu Leu Glu Arg Ser Glu Arg Ala Cys Gln 1088
1089 Glu Glu Leu Ala Gln Gln Gln Leu Leu Asp Met Ser Ser Tyr Ala Ile 1104
1105 Thr Leu Gln Gln Gln Ala Leu Asp Gly Leu Ala Ala Asp Arg Leu Ala 1120
1121 Leu Leu Ala Ser Gln Ala Thr Ala Gln Gln Arg His Asp His Tyr Tyr 1136
1137 Thr Leu Tyr Gln Asn Asn Ile Ser Ser Ala Glu Gln Leu Val Met Asp 1152
1153 Thr Gln Thr Ser Ala Gln Ser Leu Ile Ser Ser Ser Thr Gly Val Gln 1168
1169 Thr Ala Ser Gly Ala Leu Lys Val Ile Pro Asn Ile Phe Gly Leu Ala 1184
1185 Asp Gly Gly Ser Arg Tyr Glu Gly Val Thr Glu Ala Ile Ala Ile Gly 1200
1201 Leu Met Ala Ala Gly Gln Ala Thr Ser Val Val Ala Glu Arg Leu Ala 1216
1217 Thr Thr Glu Asn Tyr Arg Arg Arg Arg Glu Glu Trp Gln Ile Gln Tyr 1232
1233 Gln Gln Ala Gln Ser Glu Val Asp Ala Leu Gln Lys Gln Leu Asp Ala 1248
1249 Leu Ala Val Arg Glu Lys Ala Ala Gln Thr Ser Leu Gln Gln Ala Lys 1264
1265 Ala Gln Gln Val Gln Ile Arg Thr Met Leu Thr Tyr Leu Thr Thr Arg 1280
1281 Phe Thr Gln Ala Thr Leu Tyr Gln Trp Leu Ser Gly Gln Leu Ser Ala 1296
1297 Leu Tyr Tyr Gln Ala Tyr Asp Ala Val Val Ala Leu Cys Leu Ser Ala 1312
1313 Gln Ala Cys Trp Gln Tyr Glu Leu Gly Asp Tyr Ala Thr Thr Phe Ile 1328
1329 Gln Thr Gly Thr Trp Asn Asp His Tyr Arg Gly Leu Gln Val Gly Glu 1344
1345 Thr Leu Gln Leu Asn Leu His Gln Met Glu Ala Ala Tyr Leu Val Arg 1360
1361 His Glu Arg Arg Leu Asn Val Ile Arg Thr Val Ser Leu Lys Ser Leu 1376
1377 Leu Gly Asp Asp Gly Phe Gly Lys Leu Lys Thr Glu Gly Lys Val Asp 1392
1393 Phe Pro Leu Ser Glu Lys Leu Phe Asp Asn Asp Tyr Pro Gly His Tyr 1408
1409 Leu Arg Gln Ile Lys Thr Val Ser Val Thr Leu Pro Thr Leu Val Gly 1424
1425 Pro Tyr Gln Asn Val Lys Ala Thr Leu Thr Gln Thr Ser Ser Ser Ile 1440
1441 Leu Leu Ala Ala Asp Ile Asn Gly Val Lys Arg Leu Asn Asp Pro Thr 1456
1457 Gly Lys Glu Gly Asp Ala Thr His Ile Val Thr Asn Leu Arg Ala Ser 1472
1473 Gln Gln Val Ala Leu Ser Ser Gly Ile Asn Asp Ala Gly Ser Phe Glu 1488
1489 Leu Arg Leu Glu Asp Glu Arg Tyr Leu Ser Phe Glu Gly Thr Gly Ala 1504
1505 Val Ser Lys Trp Thr Leu Asn Phe Pro Arg Ser Val Asp Glu His Ile 1520
1521 Asp Asp Lys Thr Leu Lys Ala Asp Glu Met Gln Ala Ala Leu Leu Ala 1536
1537 Asn Met Asp Asp Val Leu Val Gln Val His Tyr Thr Ala Cys Asp Gly 1552
1553 Gly Ala Ser Phe Ala Asn Gln Val Lys Lys Thr Leu Ser 1565

(2) INFORMATION FOR SEQ ID NO:60

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3132 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60 (cccC)

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   1  ATG AGT CCG TCT GAG ACT ACT CTT TAT ACT CAA ACC CCA ACA GTC AGC   48
   1  Met Ser Pro Ser Glu Thr Thr Leu Tyr Thr Gln Thr Pro Thr Val Ser   16

  49  GTG TTA GAT AAT CGC GGT CTG TCC ATT CGT GAT ATT GGT TTT CAC CGT   96
  17  Val Leu Asp Asn Arg Gly Leu Ser Ile Arg Asp Ile Gly Phe His Arg   32

  97  ATT GTA ATC GGG GGG GAT ACT GAC ACC CGC GTC ACC CGT CAC CAG TAT  144
  33  Ile Val Ile Gly Gly Asp Thr Asp Thr Arg Val Thr Arg His Gln Tyr   48

 145  GAT GCC CGT GGA CAC CTG AAC TAC AGT ATT GAC CCA CGC TTG TAT GAT  192
  49  Asp Ala Arg Gly His Leu Asn Tyr Ser Ile Asp Pro Arg Leu Tyr Asp   64

 193  GCA AAG CAG GCT GAT AAC TCA GTA AAG CCT AAT TTT GTC TGG CAG CAT  240
  65  Ala Lys Gln Ala Asp Asn Ser Val Lys Pro Asn Phe Val Trp Gln His   80

 241  GAT CTG GCC GGT CAT GCC CTG CGG ACA GAG AGT GTC GAT GCT GGT CGT  288
  81  Asp Leu Ala Gly His Ala Leu Arg Thr Glu Ser Val Asp Ala Gly Arg   96

 289  ACT GTT GCA TTG AAT GAT ATT GAA GGT CGT TCG GTA ATG ACA ATG AAT  336
  97  Thr Val Ala Leu Asn Asp Ile Glu Gly Arg Ser Val Met Thr Met Asn  112

 337  GCG ACC GGT GTT CGT CAG ACC CGT CGC TAT GAA GGC AAC ACC TTG CCC  384
 113  Ala Thr Gly Val Arg Gln Thr Arg Arg Tyr Glu Gly Asn Thr Leu Pro  128

 385  GGT CGC TTG TTA TCT GTG AGC GAG CAA GTT TTC AAC CAA GAG AGT GCT  432
 129  Gly Arg Leu Leu Ser Val Ser Glu Gln Val Phe Asn Gln Glu Ser Ala  144

 433  AAA GTG ACA GAG CGC TTT ATC TGG GCT GGG AAT ACA ACC TCG GAG AAA  480
 145  Lys Val Thr Glu Arg Phe Ile Trp Ala Gly Asn Thr Thr Ser Glu Lys  160

 481  GAG TAT AAC CTC TCC GGT CTG TGT ATA CGC CAC TAC GAC ACA GCG GGA  528
 161  Glu Tyr Asn Leu Ser Gly Leu Cys Ile Arg His Tyr Asp Thr Ala Gly  176

 529  GTG ACC CGG TTG ATG AGT CAG TCA CTG GCG GGC GCC ATG CTA TCC CAA  576
 177  Val Thr Arg Leu Met Ser Gln Ser Leu Ala Gly Ala Met Leu Ser Gln  192

 577  TCT CAC CAA TTG CTG GCG GAA GGG CAG GAG GCT AAC TGG AGC GGT GAC  624
 193  Ser His Gln Leu Leu Ala Glu Gly Gln Glu Ala Asn Trp Ser Gly Asp  208
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625  GAC GAA ACT GTC TGG CAG GGA ATG CTG GCA AGT GAG GTC TAT ACG ACA  672
209  Asp Glu Thr Val Trp Gln Gly Met Leu Ala Ser Glu Val Tyr Thr Thr  224

673  CAA AGT ACC ACT AAT GCC ATC GGG GCT TTA CTG ACC CAA ACC GAT GCG  720
225  Gln Ser Thr Thr Asn Ala Ile Gly Ala Leu Leu Thr Gln Thr Asp Ala  240

721  AAA GGC AAT ATT CAG CGT CTG GCT TAT GAC ATT GCC GGT CAG TTA AAA  768
241  Lys Gly Asn Ile Gln Arg Leu Ala Tyr Asp Ile Ala Gly Gln Leu Lys  256

769  GGG AGT TGG TTG ACG GTG AAA GGC CAG AGT GAA CAG GTG ATT GTT AAG  816
257  Gly Ser Trp Leu Thr Val Lys Gly Gln Ser Glu Gln Val Ile Val Lys  272

817  TCC CTG AGC TGG TCA GCC GCA GGT CAT AAA TTG CGT GAA GAG CAC GGT  864
273  Ser Leu Ser Trp Ser Ala Ala Gly His Lys Leu Arg Glu Glu His Gly  288

865  AAC GGC GTG GTT ACG GAG TAC AGT TAT GAG CCG GAA ACT CAA CGT CTG  912
289  Asn Gly Val Val Thr Glu Tyr Ser Tyr Glu Pro Glu Thr Gln Arg Leu  304

913  ATA GGT ATC ACC ACC CGG CGT GCC GAA GGG AGT CAA TCA GGA GCC AGA  960
305  Ile Gly Ile Thr Thr Arg Arg Ala Glu Gly Ser Gln Ser Gly Ala Arg  320

961  GTA TTG CAG GAT CTA CGC TAT AAG TAT GAT CCG GTG GGG AAT GTT ATC 1008
321  Val Leu Gln Asp Leu Arg Tyr Lys Tyr Asp Pro Val Gly Asn Val Ile  336

1009 AGT ATC CAT AAT GAT GCC GAA GCT ACC CGC TTT TGG CGT AAT CAG AAA 1056
337  Ser Ile His Asn Asp Ala Glu Ala Thr Arg Phe Trp Arg Asn Gln Lys  352

1057 GTG GAG CCG GAG AAT CGC TAT GTT TAT GAT TCT CTG TAT CAG CTT ATG 1104
353  Val Glu Pro Glu Asn Arg Tyr Val Tyr Asp Ser Leu Tyr Gln Leu Met  368

1105 AGT GCG ACA GGG CGT GAA ATG GCT AAT ATC GGT CAG CAA AGC AAC CAA 1152
369  Ser Ala Thr Gly Arg Glu Met Ala Asn Ile Gly Gln Gln Ser Asn Gln  384

1153 CTT CCC TCA CCC GTT ATA CCT GTT CCT ACT GAC GAC AGC ACT TAT ACC 1200
385  Leu Pro Ser Pro Val Ile Pro Val Pro Thr Asp Asp Ser Thr Tyr Thr  400

1201 AAT TAC CTT CGT ACC TAT ACT TAT GAC CGT GGC GGT AAT TTG GTT CAA 1248
401  Asn Tyr Leu Arg Thr Tyr Thr Tyr Asp Arg Gly Gly Asn Leu Val Gln  416

1249 ATC CGA CAC AGT TCA CCC GCG ACT CAA AAT AGT TAC ACC ACA GAT ATC 1296
417  Ile Arg His Ser Ser Pro Ala Thr Gln Asn Ser Tyr Thr Thr Asp Ile  432

1297 ACC GTT TCA AGC CGC AGT AAC CGG GCG GTA TTG AGT ACA TTA ACG ACA 1344
433  Thr Val Ser Ser Arg Ser Asn Arg Ala Val Leu Ser Thr Leu Thr Thr  448

1345 GAT CCA ACC CGA GTG GAT GCG CTA TTT GAT TCC GGC GGT CAT CAG AAG 1392
449  Asp Pro Thr Arg Val Asp Ala Leu Phe Asp Ser Gly Gly His Gln Lys  464

1393 ATG TTA ATA CCG GGG CAA AAT CTG GAT TGG AAT ATT CGG GGT GAA TTG 1440
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465  Met Leu Ile Pro Gly Gln Asn Leu Asp Trp Asn Ile Arg Gly Glu Leu  480

1441 CAA CGA GTC ACA CCG GTG AGC CGT GAA AAT AGC AGT GAC AGT GAA TGG 1488
 481 Gln Arg Val Thr Pro Val Ser Arg Glu Asn Ser Ser Asp Ser Glu Trp  496

1489 TAT CGC TAT AGC AGT GAT GGC ATG CGG CTG CTA AAA GTG AGT GAA CAG 1536
 497 Tyr Arg Tyr Ser Ser Asp Gly Met Arg Leu Leu Lys Val Ser Glu Gln  512

1537 CAG ACG GGC AAC AGT ACT CAA GTA CAA CGG GTG ACT TAT CTG CCG GGA 1584
 513 Gln Thr Gly Asn Ser Thr Gln Val Gln Arg Val Thr Tyr Leu Pro Gly  528

1585 TTA GAG CTA CGG ACA ACT GGG GTT GCA GAT AAA ACA ACC GAA GAT TTG 1632
 529 Leu Glu Leu Arg Thr Thr Gly Val Ala Asp Lys Thr Thr Glu Asp Leu  544

1633 CAG GTG ATT ACG GTA GGT GAA GCG GGT CGC GCA CAG GTA AGG GTA TTG 1680
 545 Gln Val Ile Thr Val Gly Glu Ala Gly Arg Ala Gln Val Arg Val Leu  560

1681 CAC TGG GAA AGT GGT AAG CCG ACA GAT ATT GAC AAC AAT CAG GTG CGC 1728
 561 His Trp Glu Ser Gly Lys Pro Thr Asp Ile Asp Asn Asn Gln Val Arg  576

1729 TAC AGC TAC GAT AAT CTG CTT GGC TCC AGC CAG CTT GAA CTG GAT AGC 1776
 577 Tyr Ser Tyr Asp Asn Leu Leu Gly Ser Ser Gln Leu Glu Leu Asp Ser  592

1777 GAA GGG CAG ATT CTC AGT CAG GAA GAG TAT TAT CCG TAT GGC GGT ACG 1824
 593 Glu Gly Gln Ile Leu Ser Gln Glu Glu Tyr Tyr Pro Tyr Gly Gly Thr  608

1825 GCG ATA TGG GCG GCG AGA AAT CAG ACA GAA GCC AGC TAC AAA TTT ATT 1872
 609 Ala Ile Trp Ala Ala Arg Asn Gln Thr Glu Ala Ser Tyr Lys Phe Ile  624

1873 CGT TAC TCC GGT AAA GAG CGG GAT GCC ACT GGA TTG TAT TAT TAC GGC 1920
 625 Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly Leu Tyr Tyr Tyr Gly  640

1921 TAC CGT TAT TAT CAA CCT TGG GTG GGT CGA TGG TTG AGT GCT GAT CCG 1968
 641 Tyr Arg Tyr Tyr Gln Pro Trp Val Gly Arg Trp Leu Ser Ala Asp Pro  656

1969 GCG GGA ACC GTG GAT GGG CTG AAT TTG TAC CGA ATG GTG AGG AAT AAC 2016
 657 Ala Gly Thr Val Asp Gly Leu Asn Leu Tyr Arg Met Val Arg Asn Asn  672

2017 CCC ATC ACA TTG ACT GAC CAT GAC GGA TTA GCA CCG TCT CCA AAT AGA 2064
 673 Pro Ile Thr Leu Thr Asp His Asp Gly Leu Ala Pro Ser Pro Asn Arg  688

2065 AAT CGA AAT ACA TTT TGG TTT GCT TCA TTT TTG TTT CGT AAA CCT GAT 2112
 689 Asn Arg Asn Thr Phe Trp Phe Ala Ser Phe Leu Phe Arg Lys Pro Asp  704

2113 GAG GGA ATG TCC GCG TCA ATG AGA CGG GGA CAA AAA ATT GGC AGA GCC 2160
 705 Glu Gly Met Ser Ala Ser Met Arg Arg Gly Gln Lys Ile Gly Arg Ala  720

2161 ATT GCC GGC GGG ATT GCG ATT GGC GGT CTT GCG GCT ACC ATT GCC GCT 2208
 721 Ile Ala Gly Gly Ile Ala Ile Gly Gly Leu Ala Ala Thr Ile Ala Ala  736
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2209  ACG GCT GGC GCG GCT ATC CCC GTC ATT CTG GGG GTT GCG GCC GTA GGC  2256
 737  Thr Ala Gly Ala Ala Ile Pro Val Ile Leu Gly Val Ala Ala Val Gly   752

2257  GCG GGG ATT GGC GCG TTG ATG GGA TAT AAC GTC GGT AGC CTG CTG GAA  2304
 753  Ala Gly Ile Gly Ala Leu Met Gly Tyr Asn Val Gly Ser Leu Leu Glu   768

2305  AAA GGC GGG GCA TTA CTT GCT CGA CTC GTA CAG GGG AAA TCG ACG TTA  2352
 769  Lys Gly Gly Ala Leu Leu Ala Arg Leu Val Gln Gly Lys Ser Thr Leu   784

2353  GTA CAG TCG GCG GCT GGC GCG GCT GCC GGA GCG AGT TCA GCC GCG GCT  2400
 785  Val Gln Ser Ala Ala Gly Ala Ala Ala Gly Ala Ser Ser Ala Ala Ala   800

2401  TAT GGC GCA CGG GCA CAA GGT GTC GGT GTT GCA TCA GCC GCC GGG GCG  2448
 801  Tyr Gly Ala Arg Ala Gln Gly Val Gly Val Ala Ser Ala Ala Gly Ala   816

2449  GTA ACA GGG GCT GTG GGA TCA TGG ATA AAT AAT GCT GAT CGG GGG ATT  2496
 817  Val Thr Gly Ala Val Gly Ser Trp Ile Asn Asn Ala Asp Arg Gly Ile   832

2497  GGC GGC GCT ATT GGG GCC GGG AGT GCG GTA GGC ACC ATT GAT ACT ATG  2544
 833  Gly Gly Ala Ile Gly Ala Gly Ser Ala Val Gly Thr Ile Asp Thr Met   848

2545  TTA GGG ACT GCC TCT ACC CTT ACC CAT GAA GTC GGG GCA GCG GCG GGT  2592
 849  Leu Gly Thr Ala Ser Thr Leu Thr His Glu Val Gly Ala Ala Ala Gly   864

2593  GGG GCG GCG GGT GGG ATG ATC ACC GGT ACG CAA GGG AGT ACT CGG GCA  2640
 865  Gly Ala Ala Gly Gly Met Ile Thr Gly Thr Gln Gly Ser Thr Arg Ala   880

2641  GGT ATC CAT GCC GGT ATT GGC ACC TAT TAT GGC TCC TGG ATT GGT TTT  2688
 881  Gly Ile His Ala Gly Ile Gly Thr Tyr Tyr Gly Ser Trp Ile Gly Phe   896

2689  GGT TTA GAT GTC GCT AGT AAC CCC GCC GGA CAT TTA GCG AAT TAC GCA  2736
 897  Gly Leu Asp Val Ala Ser Asn Pro Ala Gly His Leu Ala Asn Tyr Ala   912

2737  GTG GGT TAT GCC GCT GGT TTG GGT GCT GAA ATG GCT GTC AAC AGA ATA  2784
 913  Val Gly Tyr Ala Ala Gly Leu Gly Ala Glu Met Ala Val Asn Arg Ile   928

2785  ATG GGT GGT GGA TTT TTG AGT AGG CTC TTA GGC CGG GTT GTC AGC CCA  2832
 929  Met Gly Gly Gly Phe Leu Ser Arg Leu Leu Gly Arg Val Val Ser Pro   944

2833  TAT GCC GCC GGT TTA GCC AGA CAA TTA GTA CAT TTC AGT GTC GCC AGA  2880
 945  Tyr Ala Ala Gly Leu Ala Arg Gln Leu Val His Phe Ser Val Ala Arg   960

2881  CCT GTC TTT GAG CCG ATA TTT AGT GTT CTC GGC GGG CTT GTC GGT GGT  2928
 961  Pro Val Phe Glu Pro Ile Phe Ser Val Leu Gly Gly Leu Val Gly Gly   976

2929  ATT GGA ACT GGC CTG CAC AGA GTG ATG GGA AGA GAG AGT TGG ATT TCC  2976
 977  Ile Gly Thr Gly Leu His Arg Val Met Gly Arg Glu Ser Trp Ile Ser   992

2977  AGA GCG TTA AGT GCT GCC GGT AGT GGT ATA GAT CAT GTC GCT GGC ATG  3024
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993 Arg Ala Leu Ser Ala Ala Gly Ser Gly Ile Asp His Val Ala Gly Met 1013

3025 ATT GGT AAT CAG ATC AGA GGC AGG GTC TTG ACC ACA ACC GGG ATC GCT 3072
1009 Ile Gly Asn Gln Ile Arg Gly Arg Val Leu Thr Thr Thr Gly Ile Ala 1024

3073 AAT GCG ATA GAC TAT GGC ACC AGT GCT GTG GGA GCC GCA CGA CGA GTT 3120
1025 Asn Ala Ile Asp Tyr Gly Thr Ser Ala Val Gly Ala Ala Arg Arg Val 1040

3121 TTT TCT TTG TAA 3132
1041 Phe Ser Leu End 1043

(2) INFORMATION FOR SEQ ID NO:61
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1043 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61 (TccC peptide)

1 Met Ser Pro Ser Glu Thr Thr Leu Tyr Thr Gln Thr Pro Thr Val Ser 16
17 Val Leu Asp Asn Arg Gly Leu Ser Ile Arg Asp Ile Gly Phe His Arg 32
33 Ile Val Ile Gly Gly Asp Thr Asp Thr Arg Val Thr Arg His Gln Tyr 48
49 Asp Ala Arg Gly His Leu Asn Tyr Ser Ile Asp Pro Arg Leu Tyr Asp 64
65 Ala Lys Gln Ala Asp Asn Ser Val Lys Pro Asn Phe Val Trp Gln His 80
81 Asp Leu Ala Gly His Ala Leu Arg Thr Glu Ser Val Asp Ala Gly Arg 96
97 Thr Val Ala Leu Asn Asp Ile Glu Gly Arg Ser Val Met Thr Met Asn 112
113 Ala Thr Gly Val Arg Gln Thr Arg Arg Tyr Glu Gly Asn Thr Leu Pro 128
129 Gly Arg Leu Leu Ser Val Ser Glu Gln Val Phe Asn Gln Glu Ser Ala 144
145 Lys Val Thr Glu Arg Phe Ile Trp Ala Gly Asn Thr Thr Ser Glu Lys 160
161 Glu Tyr Asn Leu Ser Gly Leu Cys Ile Arg His Tyr Asp Thr Ala Gly 176
177 Val Thr Arg Leu Met Ser Gln Ser Leu Ala Gly Ala Met Leu Ser Gln 192
193 Ser His Gln Leu Leu Ala Glu Gly Gln Glu Ala Asn Trp Ser Gly Asp 208
209 Asp Glu Thr Val Trp Gln Gly Met Leu Ala Ser Glu Val Tyr Thr Thr 224
225 Gln Ser Thr Thr Asn Ala Ile Gly Ala Leu Leu Thr Gln Thr Asp Ala 240
241 Lys Gly Asn Ile Gln Arg Leu Ala Tyr Asp Ile Ala Gly Gln Leu Lys 256
257 Gly Ser Trp Leu Thr Val Lys Gly Gln Ser Glu Gln Val Ile Val Lys 272
273 Ser Leu Ser Trp Ser Ala Ala Gly His Lys Leu Arg Glu Glu His Gly 288
289 Asn Gly Val Val Thr Glu Tyr Ser Tyr Glu Pro Glu Thr Gln Arg Leu 304
305 Ile Gly Ile Thr Thr Arg Arg Ala Glu Gly Ser Gln Ser Gly Ala Arg 320

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|---|---|---|---|
| 321 | Val Leu Gln Asp Leu Arg Tyr Lys Tyr Asp Pro Val Gly Asn Val Ile | | 336 |
| 337 | Ser Ile His Asn Asp Ala Glu Ala Thr Arg Phe Trp Arg Asn Gln Lys | | 352 |
| 353 | Val Glu Pro Glu Asn Arg Tyr Val Tyr Asp Ser Leu Tyr Gln Leu Met | | 368 |
| 369 | Ser Ala Thr Gly Arg Glu Met Ala Asn Ile Gly Gln Gln Ser Asn Gln | | 384 |
| 385 | Leu Pro Ser Pro Val Ile Pro Val Pro Thr Asp Asp Ser Thr Tyr Thr | | 400 |
| 401 | Asn Tyr Leu Arg Thr Tyr Thr Tyr Asp Arg Gly Gly Asn Leu Val Gln | | 416 |
| 417 | Ile Arg His Ser Ser Pro Ala Thr Gln Asn Ser Tyr Thr Thr Asp Ile | | 432 |
| 433 | Thr Val Ser Ser Arg Ser Asn Arg Ala Val Leu Ser Thr Leu Thr Thr | | 448 |
| 449 | Asp Pro Thr Arg Val Asp Ala Leu Phe Asp Ser Gly Gly His Gln Lys | | 464 |
| 465 | Met Leu Ile Pro Gly Gln Asn Leu Asp Trp Asn Ile Arg Gly Glu Leu | | 480 |
| 481 | Gln Arg Val Thr Pro Val Ser Arg Glu Asn Ser Ser Asp Ser Glu Trp | | 496 |
| 497 | Tyr Arg Tyr Ser Ser Asp Gly Met Arg Leu Leu Lys Val Ser Glu Gln | | 512 |
| 513 | Gln Thr Gly Asn Ser Thr Gln Val Gln Arg Val Thr Tyr Leu Pro Gly | | 528 |
| 529 | Leu Glu Leu Arg Thr Thr Gly Val Ala Asp Lys Thr Thr Glu Asp Leu | | 544 |
| 545 | Gln Val Ile Thr Val Gly Glu Ala Gly Arg Ala Gln Val Arg Val Leu | | 560 |
| 561 | His Trp Glu Ser Gly Lys Pro Thr Asp Ile Asp Asn Asn Gln Val Arg | | 576 |
| 577 | Tyr Ser Tyr Asp Asn Leu Leu Gly Ser Ser Gln Leu Glu Leu Asp Ser | | 592 |
| 593 | Glu Gly Gln Ile Leu Ser Gln Glu Glu Tyr Tyr Pro Tyr Gly Gly Thr | | 608 |
| 609 | Ala Ile Trp Ala Ala Arg Asn Gln Thr Glu Ala Ser Tyr Lys Phe Ile | | 624 |
| 625 | Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly Leu Tyr Tyr Tyr Gly | | 640 |
| 641 | Tyr Arg Tyr Tyr Gln Pro Trp Val Gly Arg Trp Leu Ser Ala Asp Pro | | 656 |
| 657 | Ala Gly Thr Val Asp Gly Leu Asn Leu Tyr Arg Met Val Arg Asn Asn | | 672 |
| 673 | Pro Ile Thr Leu Thr Asp His Asp Gly Leu Ala Pro Ser Pro Asn Arg | | 688 |
| 689 | Asn Arg Asn Thr Phe Trp Phe Ala Ser Phe Leu Phe Arg Lys Pro Asp | | 704 |
| 705 | Glu Gly Met Ser Ala Ser Met Arg Arg Gly Gln Lys Ile Gly Arg Ala | | 720 |
| 721 | Ile Ala Gly Gly Ile Ala Ile Gly Gly Leu Ala Ala Thr Ile Ala Ala | | 736 |
| 737 | Thr Ala Gly Ala Ala Ile Pro Val Ile Leu Gly Val Ala Ala Val Gly | | 752 |
| 753 | Ala Gly Ile Gly Ala Leu Met Gly Tyr Asn Val Gly Ser Leu Leu Glu | | 768 |
| 769 | Lys Gly Gly Ala Leu Leu Ala Arg Leu Val Gln Gly Lys Ser Thr Leu | | 784 |
| 785 | Val Gln Ser Ala Ala Gly Ala Ala Ala Gly Ala Ser Ser Ala Ala Ala | | 800 |
| 801 | Tyr Gly Ala Arg Ala Gln Gly Val Gly Val Ala Ser Ala Ala Gly Ala | | 816 |
| 817 | Val Thr Gly Ala Val Gly Ser Trp Ile Asn Asn Ala Asp Arg Gly Ile | | 832 |
| 833 | Gly Gly Ala Ile Gly Ala Gly Ser Ala Val Gly Thr Ile Asp Thr Met | | 848 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 849 | Leu | Gly | Thr | Ala | Ser | Thr | Leu | Thr | His | Glu | Val | Gly | Ala | Ala | Ala | Gly | 864 |
| 865 | Gly | Ala | Ala | Gly | Gly | Met | Ile | Thr | Gly | Thr | Gln | Gly | Ser | Thr | Arg | Ala | 880 |
| 881 | Gly | Ile | His | Ala | Gly | Ile | Gly | Thr | Tyr | Tyr | Gly | Ser | Trp | Ile | Gly | Phe | 896 |
| 897 | Gly | Leu | Asp | Val | Ala | Ser | Asn | Pro | Ala | Gly | His | Leu | Ala | Asn | Tyr | Ala | 912 |
| 913 | Val | Gly | Tyr | Ala | Ala | Gly | Leu | Gly | Ala | Glu | Met | Ala | Val | Asn | Arg | Ile | 928 |
| 929 | Met | Gly | Gly | Gly | Phe | Leu | Ser | Arg | Leu | Leu | Gly | Arg | Val | Val | Ser | Pro | 944 |
| 945 | Tyr | Ala | Ala | Gly | Leu | Ala | Arg | Gln | Leu | Val | His | Phe | Ser | Val | Ala | Arg | 960 |
| 961 | Pro | Val | Phe | Glu | Pro | Ile | Phe | Ser | Val | Leu | Gly | Gly | Leu | Val | Gly | Gly | 976 |
| 977 | Ile | Gly | Thr | Gly | Leu | His | Arg | Val | Met | Gly | Arg | Glu | Ser | Trp | Ile | Ser | 992 |
| 993 | Arg | Ala | Leu | Ser | Ala | Ala | Gly | Ser | Gly | Ile | Asp | His | Val | Ala | Gly | Met | 1008 |
| 1009 | Ile | Gly | Asn | Gln | Ile | Arg | Gly | Arg | Val | Leu | Thr | Thr | Thr | Gly | Ile | Ala | 1024 |
| 1025 | Asn | Ala | Ile | Asp | Tyr | Gly | Thr | Ser | Ala | Val | Gly | Ala | Ala | Arg | Arg | Val | 1040 |
| 1041 | Phe | Ser | Leu | 1043 | | | | | | | | | | | | | |

We claim:

1. A composition, comprising an effective amount of a *Photorhabdus* protein toxin that has functional activity against an insect.

2. The composition of Claim 1, wherein the *Photorhabdus* toxin is produced by a purified culture of *Photorhabdus*, a transgenic plant, Baculovirus, or heterologous microbial host.

3. The composition of Claim 2, wherein the *Photorhabdus* toxin produced by a purified culture of *Photorhabdus luminescens*.

4. The composition of Claim 2, wherein the toxin is produced from a purified culture of *Photorhabdus luminescens* strain designated ATCC 55397.

5. The composition of Claim 2, wherein the toxin is produced by a purified culture of *Photorhabdus luminescens* strain designated W-14.

6. The composition of Claim 1, wherein the toxin is produced by a purified culture of *Photorhabdus* strain designated WX-1, WX-2, WX-3, WX-4, WX-5, WX6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, ATCC# 43948, ATCC# 43949, ATCC# 43950, ATCC# 43951, or ATCC# 43952.

7. The composition of Claim 2, wherein the toxin is produced from a purified culture of *Photorhabdus luminescens* strain designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, ATCC# 43948, ATCC# 43949, ATCC# 43950, ATCC# 43951, or ATCC# 43952.

8. The composition of Claim 1, wherein the toxin is respresented by amino acid sequence is SEQ ID NO:12.

9. The composition of Claim 6, wherein the composition is a mixture of one or more toxins produced from purified cultures of *Photorhabdus*.

10. The composition of Claim 1 or 6, wherein the insect is of the order *Lepidoptera*, *Coleoptera*, *Hymenoptera*, *Diptera*, *Dictyoptera*, *Acarina* or *Homoptera*.

11. The composition of Claim 1 or 6, wherein the insect species is from order *Coleoptera* and is Southern Corn Rootworm, Western Corn Rootworm, Colorado Potato Beetle, Mealworm, Boll Weevil or Turf Grub.

12. The composition of Claim 1 or 6, wherein the insect species is from order *Lepidoptera* and is Beet Armyworm, Black Cutworm, Cabbage Looper, Codling Moth, Corn Earworm, European Corn Borer, Tobacco Hornworm, or Tobacco Budworm.

13. The composition of Claim 1 or 6, wherein the toxin is formulated as a sprayable insecticide.

14. The composition of Claim 1 or Claim 6, wherein the toxin is formulated as a bait matrix and delivered in an above ground or below ground bait station.

15. A method of controlling an insect, comprising orally delivering to an insect an effective amount of a protein toxin that has functional activity against an insect, wherein the protein is produced by a purified bacterial culture of the genus *Photorhabdus*.

16. The method of Claim 15, wherein the bacterium is a purified culture of *Photorhabdus luminescens*.

17. The method of Claim 15, wherein the toxin is produced from a purified culture of *Photorhabdus luminescens* strain designated ATCC 55397.

18. The method of Claim 16, wherein the toxin is produced from a purified culture of *Photorhabdus luminescens* strain designated W-14.

19. The method of Claim 15, wherein the toxin is produced from a purified culture of *Photorhabdus* strains designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, ATCC# 43948, ATCC# 43949, ATCC# ATCC# 43950, ATCC# 43951, or ATCC# 43952.

20. The method of Claim 15, wherein the toxin is produced from a purified culture of *Photorhabdus luminescens* strains designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, ATCC# 43948, ATCC# 43949, ATCC# ATCC# 43950, ATCC# 43951, or ATCC# 43952.

21. The method of Claim 19, wherein a mixture of one or more toxins is produced from a purified culture of *Photorhabdus* and said toxins are orally delivered to an insect.

22. The method of Claim 15, wherein the toxin is produced by a prokaryotic host transformed with a gene encoding the toxin.

23. The method of Claim 15, wherein the toxin is produced by a eukaryotic host transformed with a gene encoding the toxin.

24. The method of Claim 23, wherein the eukaryotic host is baculovirus.

25. The method of Claim 15 or 19, wherein the insect is of the order *Lepidoptera*, *Coleoptera*, *Hymenoptera*, *Diptera*, *Dictyoptera*, *Acarina* or *Homoptera*.

26. The method of Claim 15 or 19, wherein the insect species is from order *Coleoptera* and is Southern Corn Rootworm, Western Corn Rootworm, Colorado Potato Beetle, Mealworm, Boll Weevil or Turf Grub.

27. The method of Claim 15 or 19, wherein the insect species is from order *Lepidoptera* and is Beet Armyworm, Black Cutworm, Cabbage Looper, Codling Moth, Corn Earworm, European Corn Borer, Tobacco Hornworm, or Tobacco Budworm.

28. The method of Claim 15 or 19, wherein the toxin is formulated as a sprayable insecticide.

29. The method of Claim 15 or Claim 19, wherein the toxin is formulated as a bait matrix and delivered in an above ground or below ground bait station.

30. A method of isolating a gene coding for a protein subunit, comprising the steps of: constructing at least one RNA or DNA oligonucleotide molecule that corresponds to at least a part of a DNA coding region of an amino acid sequence selected from a group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43, wherein the nucleotide molecule is used to isolate genetic material from *Photorhabdus* or *Photorhabdus luminescens*.

31. A method for expressing a protein produced by a purified bacterial culture of the genus *Photorhabdus* in a prokaryotic or eukaryotic host in an effective amount so that the protein has functional activity against an insect, wherein the method comprises: constructing a chimeric DNA construct having 5' to 3' a promoter, a DNA sequence encoding a protein, a transcription terminator, and then transferring the chimeric DNA construct into the host.

32. The method of Claim 31, wherein the protein has functional activity against insects selected from a group consisting of *Coleoptera*, *Lepidoptera*, *Diptera*, *Homoptera*, *Hymenoptera*, *Dictyoptera*, and *Acarina*.

33. The method of Claim 31, wherein the protein encoded by the DNA sequence has an N-terminal amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:14, SEQ

ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

34. The method of Claim 31, wherein the protein encoded by the DNA sequence includes the amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59 and SEQ ID NO:61.

35. A chimeric DNA construct, adapted for expression in a prokaryotic or eukaryotic host comprising, 5' to 3' a transcriptional promoter active in the host; a DNA sequence encoding a *Photorhabdus* protein that has functional activity against an insect; and a transcriptional terminator.

36. A chimeric DNA construct of Claim 35, wherein the protein encoded by the DNA sequence has an N-terminal amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

37. The chimeric DNA construct of Claim 35, wherein the protein encoded by the DNA sequence has an amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, and SEQ ID NO:61.

38. The chimeric DNA construct of Claim 35, wherein the DNA sequence encoding the *Photorhabdus luminescens* protein is selected from the group comprising SEQ ID NO:11, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID

NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, and SEQ ID NO:60.

39. The chimeric DNA construct of Claim 35, wherein the host is baculovirus.

40. An isolated and substantially purified preparation comprising, a DNA molecule capable of encoding an effective amount of a protein that is produced by a bacterium of the genus *Photorhabdus* and that has functional activity against an insect.

41. The preparation of Claim 40, wherein the bacterium is *Photorhabdus luminescens*.

42. A purified preparation comprising, a protein produced by *Photorhabdus* or *Photorhabdus luminescens* having an N-terminal amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

43. A purified protein preparation comprising, a protein that has an N-terminal amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10,SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

44. A purified protein preparation comprising, a protein selected from the group of SEQ ID NO:12, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, and SEQ ID NO:61.

45. A purified DNA preparation comprising, a DNA sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58 and SEQ ID NO:60, wherein the DNA sequence is isolated from its native host.

46. A purified protein preparation comprising, a *Photorhabdus luminescens* protein with at least one subunit having an approximate molecular weight between 18 kDa to about 230 kDa; between about 160 kDa to about 230 kDa; 100 kDa to 160 kDa; about 80 kDa to about 100 kDa; or about 50 kDa to about 80 kDa.

47. A purified protein preparation comprising, a *Photorhabdus luminescens* protein with at least one subunit having an approximate molecular weight of about 280 kDa.

48. A substantially pure microorganism culture comprising, ATCC 55397.

49. The culture of Claim 48, wherein the culture is a derivative of ATCC 55397 that produces a protein toxin that has functional activity against an insect.

50. A substantially pure microorganism culture comprising, H9.

51. A substantially pure microorganism culture comprising, Hb.

52. A substantially pure microorganism culture comprising, Hm.

53. A substantially pure microorganism culture comprising, HP88.

54. A substantially pure microorganism culture comprising, NC-1.

55. A substantially pure microorganism culture comprising,

W30.

56. A substantially pure microorganism culture comprising, WIR.

57. A transgenic plant comprising in its genome, a chimeric artificial gene construction imbuing the plant with an ability to express an effective amount of a *Photorhabdus* protein that has functional activity against an insect.

58. The transgenic plant of Claim 57, wherein the plant is transformed using acceleration of genetic material coated onto microparticles directly into cells, *Agrobacteria*, whiskers, or electroporation techniques

59. The transgenic plant of Claim 57, wherein the selectable marker is selected from the group consisting of kanamycin, neomycin, glyphosate, hygromycin, methotrexate, phosphinothricin (bialophos), chlorosulfuron, bromoxynil, dalapon and the like.

60. The transgenic plant of Claim 57, wherein the promoter is selected from the group consisting of octopine synthase, nopaline synthase, mannopine synthase, 35S, 19S, ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu), beta-conglycinin, phaseolin, alcohol dehydrogenase (ADH), heat-shock, ubiquitin, zein, oleosin, napin, or acyl carier protein (ACP).

61. The transgenic plant of Claim 57, wherein embryogenic tissue, callus tissue type I or II, hypocotyl, meristem, or plant tissue during dedifferentiation is used in preparing the transgenic plant.

62. The transgenic plant of Claim 57, wherein the chimeric gene is a DNA sequence which encodes a *Photorhabdus* protein that has functional activity against an insect and at least one codon of the gene has been modified so that the codon is a plant preferred codon.

63. A method of controlling an insect comprising orally delivering to an insect an effective amount of a protein toxin, wherein the protein is produced by a transgenic plant, which said insect feeds.

64. A composition of matter, comprising a purified DNA sequence from a purified bacterial culture from the genus *Photorhabdus*.

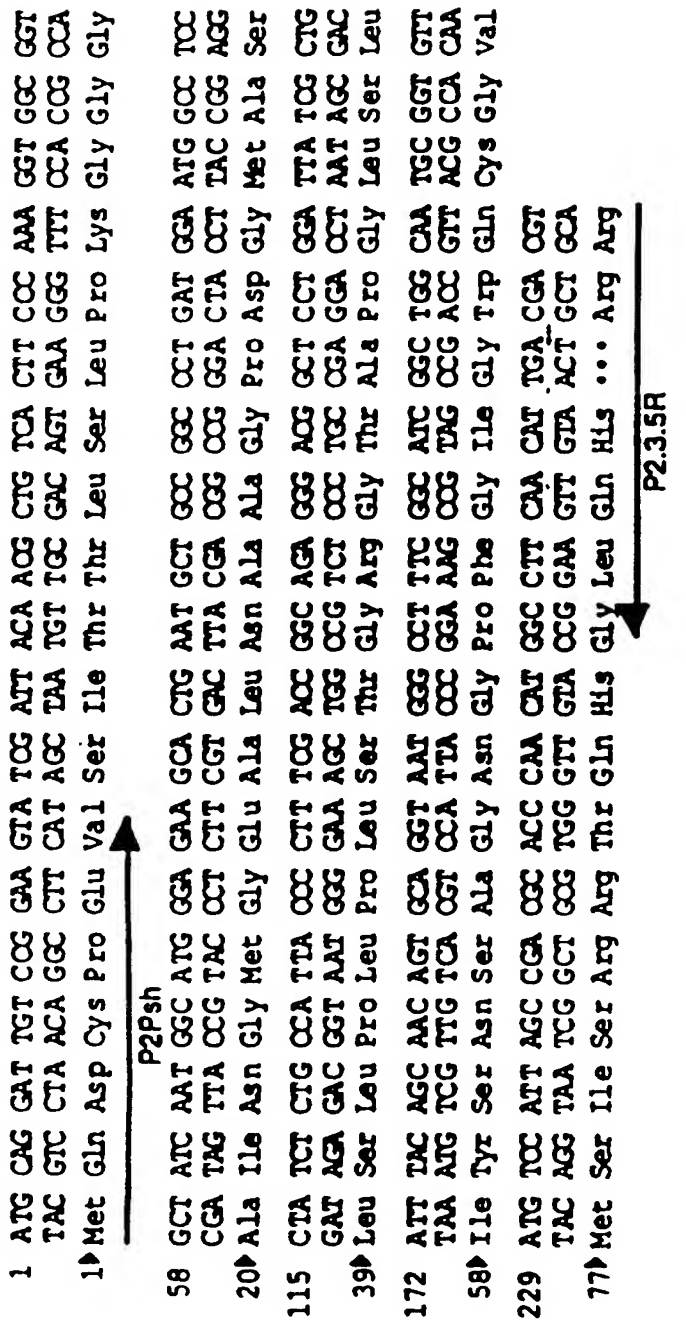



FIG. 1

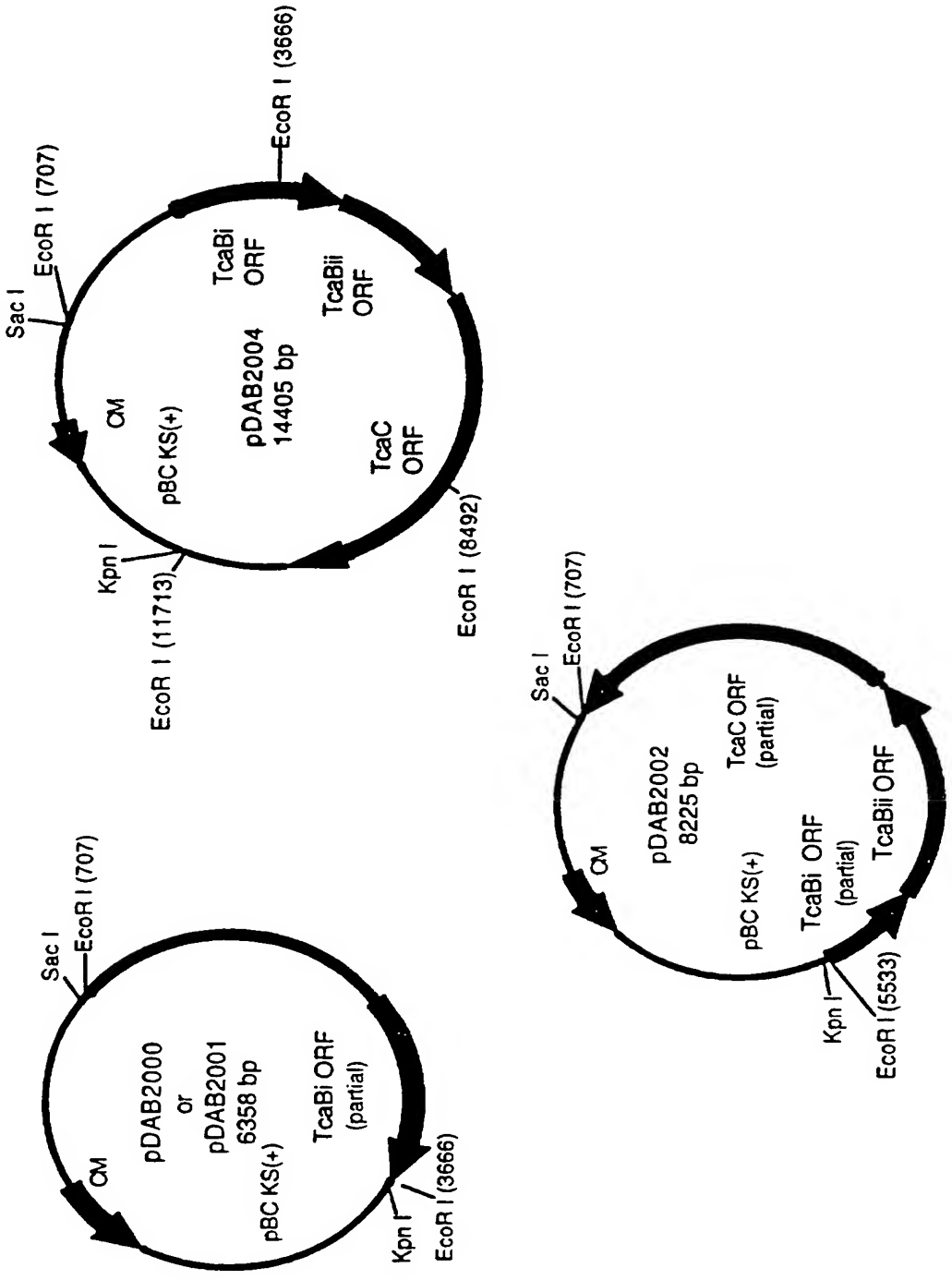



FIG. 2

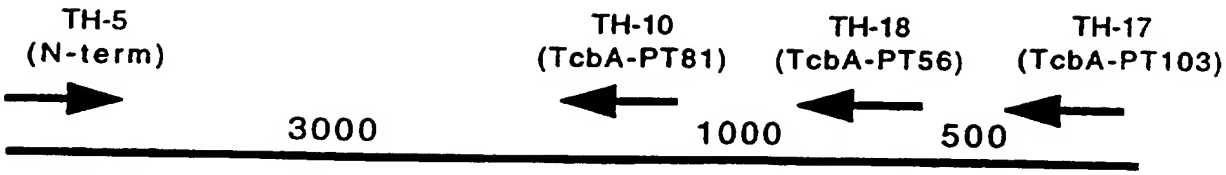
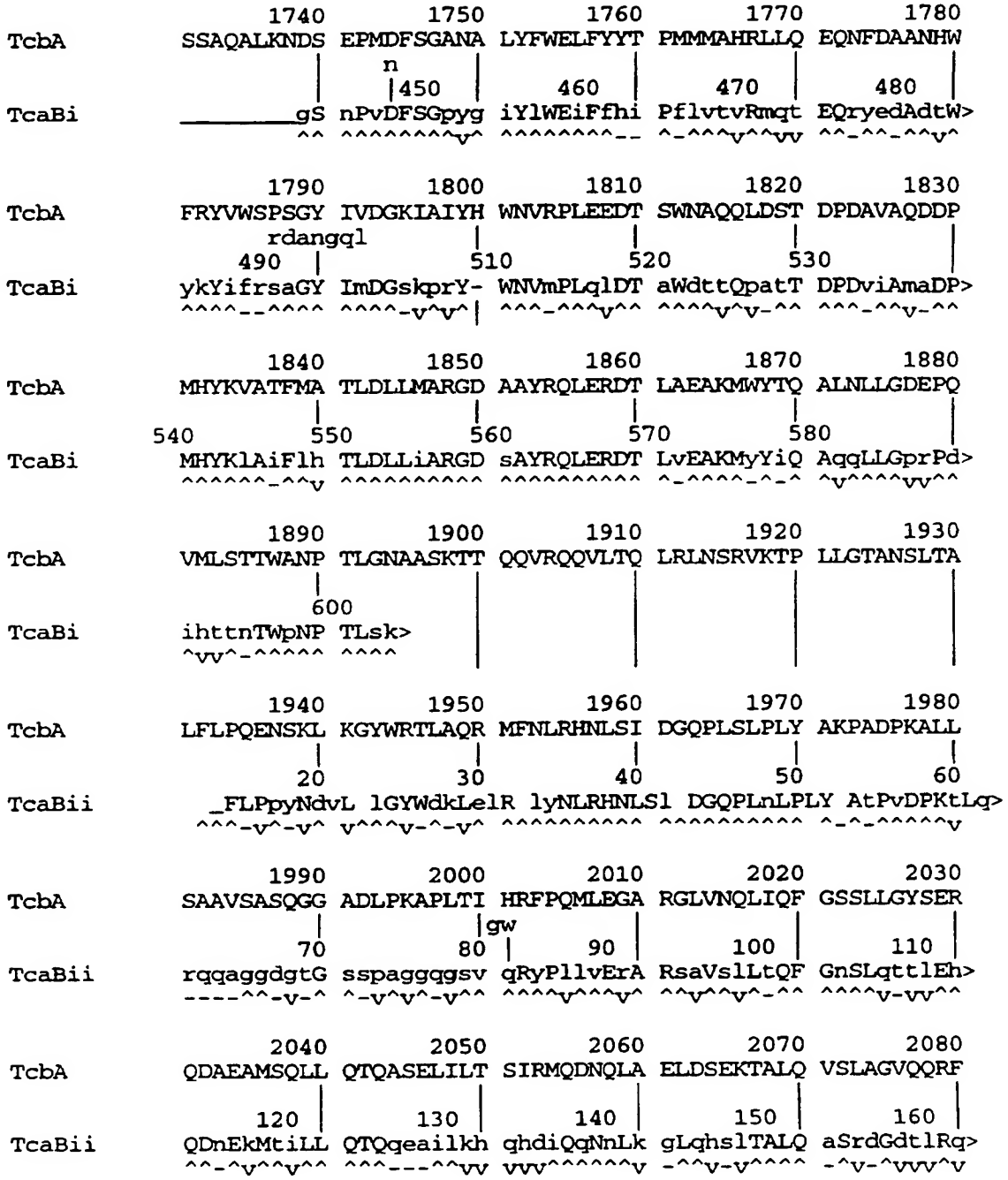



FIG. 3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490 510 520 530
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1840 1850 1860 1870 1880
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FIG. 4A

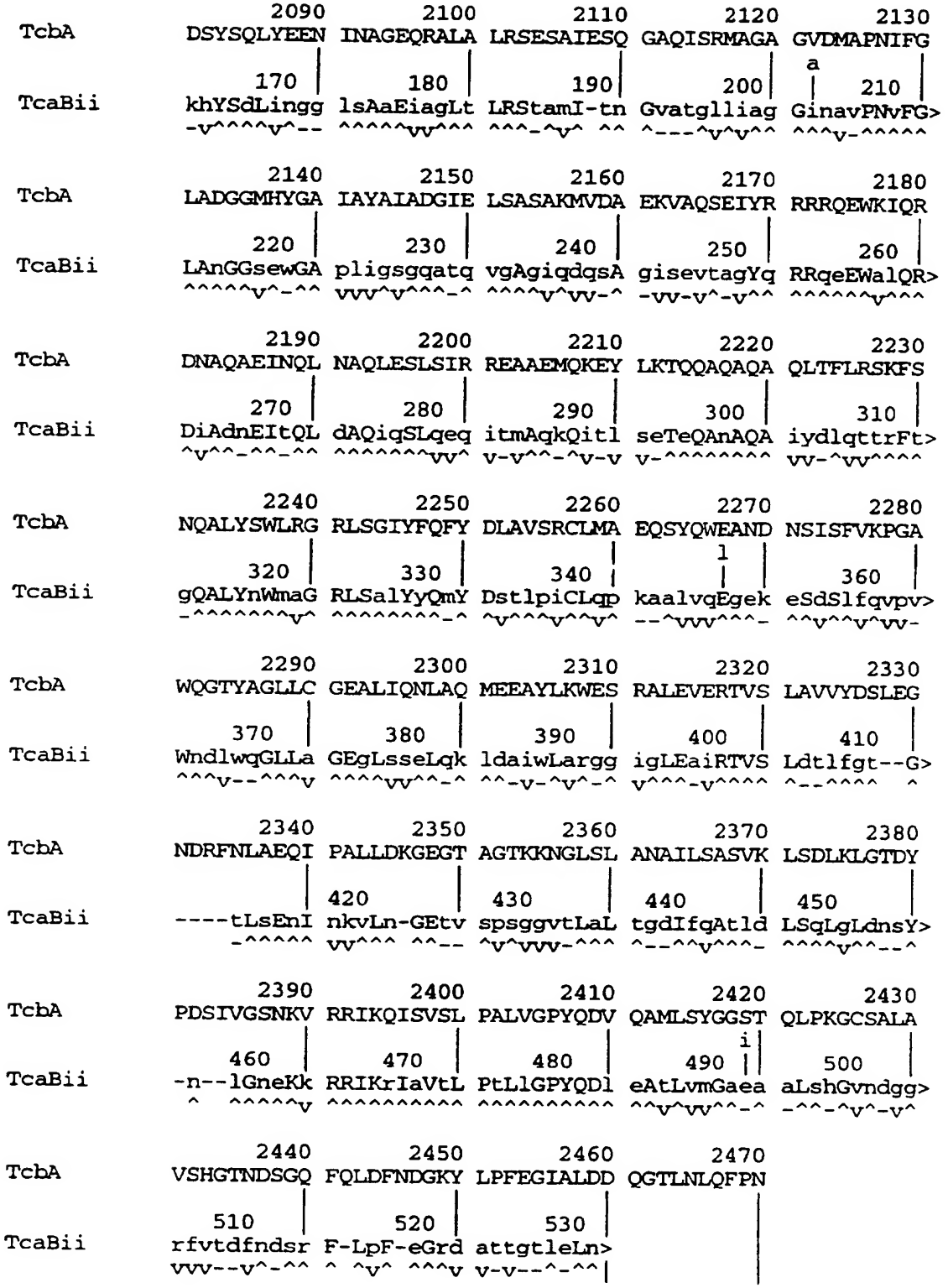


FIG. 4B

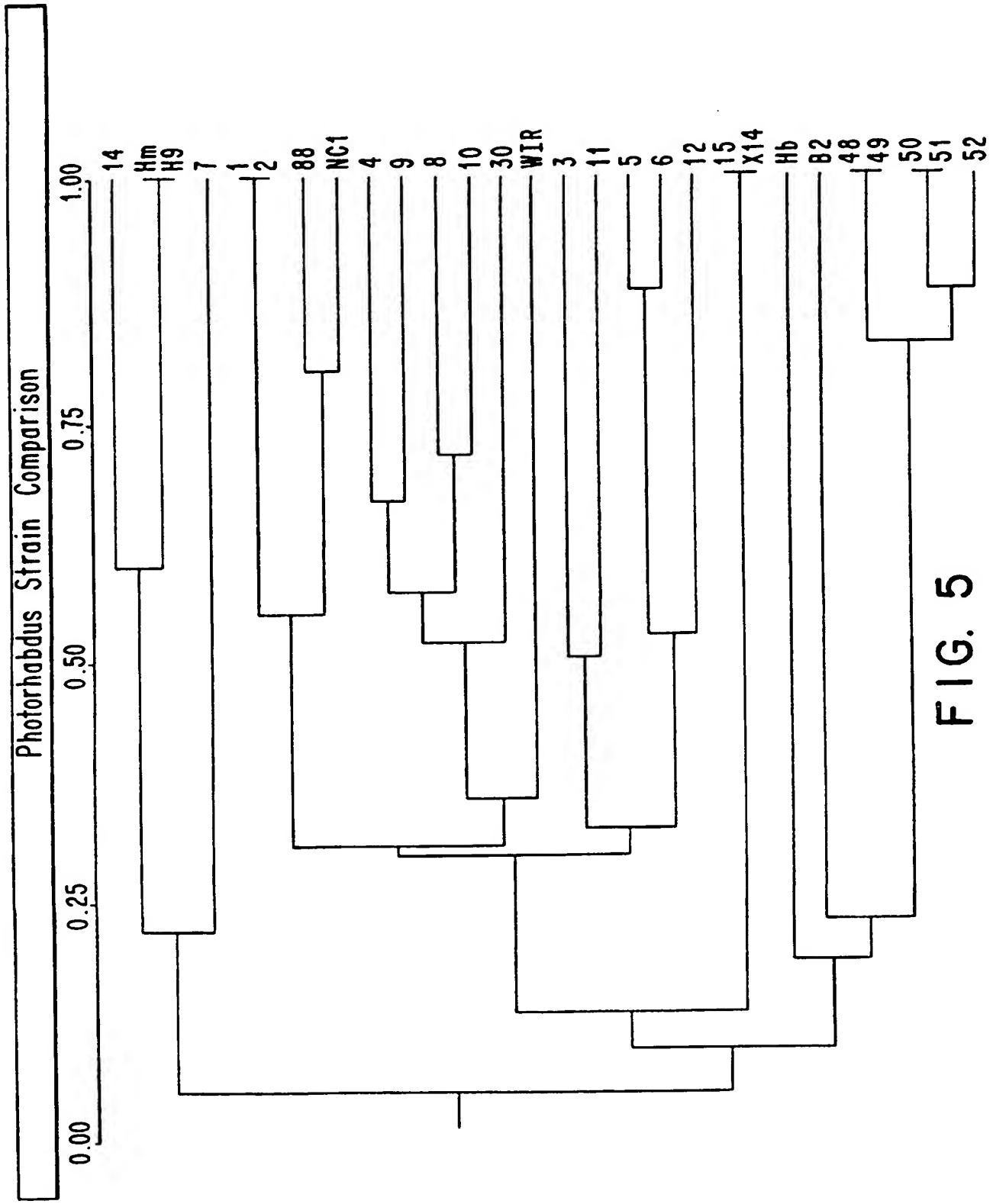


FIG. 5

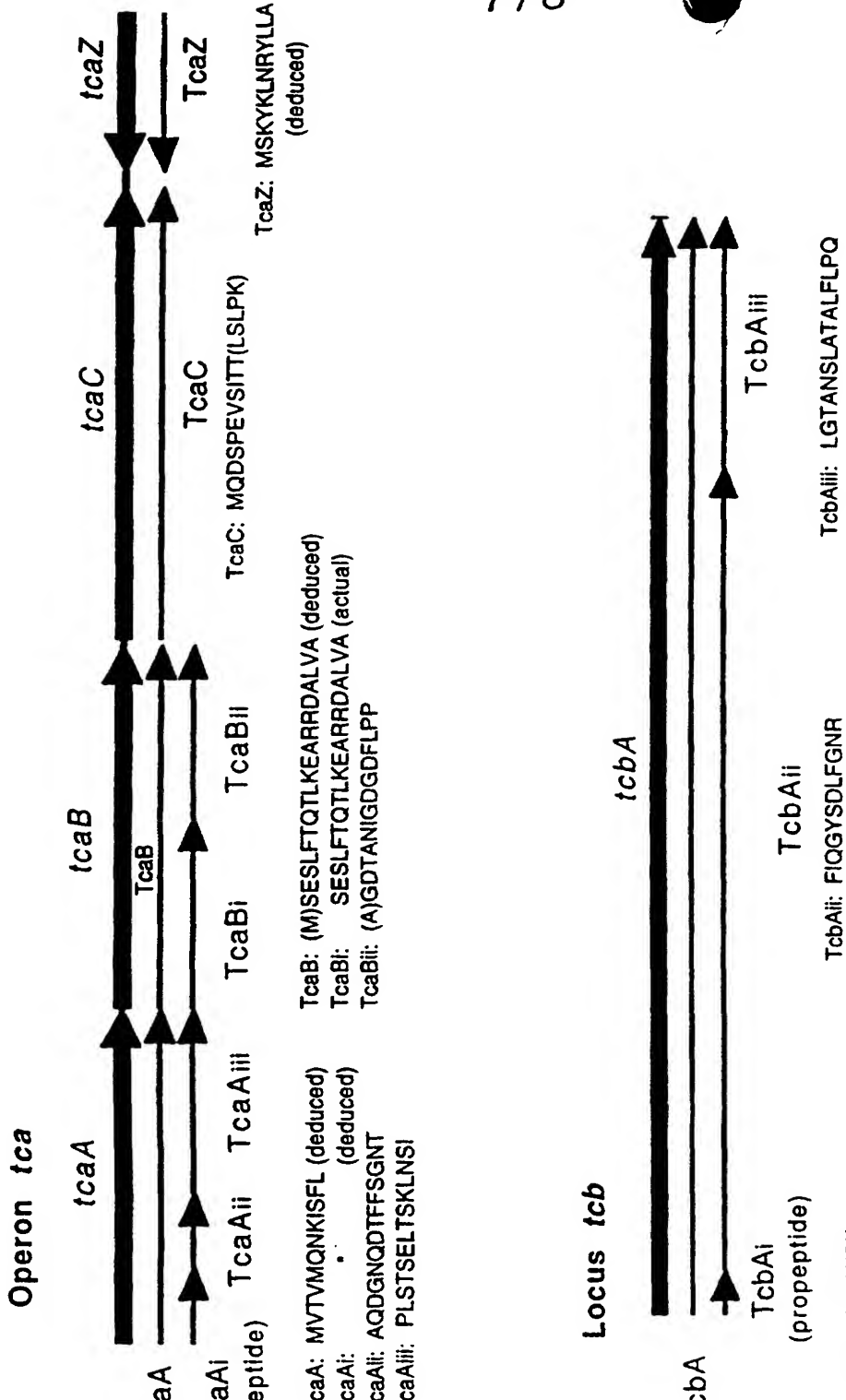


FIG. 6A

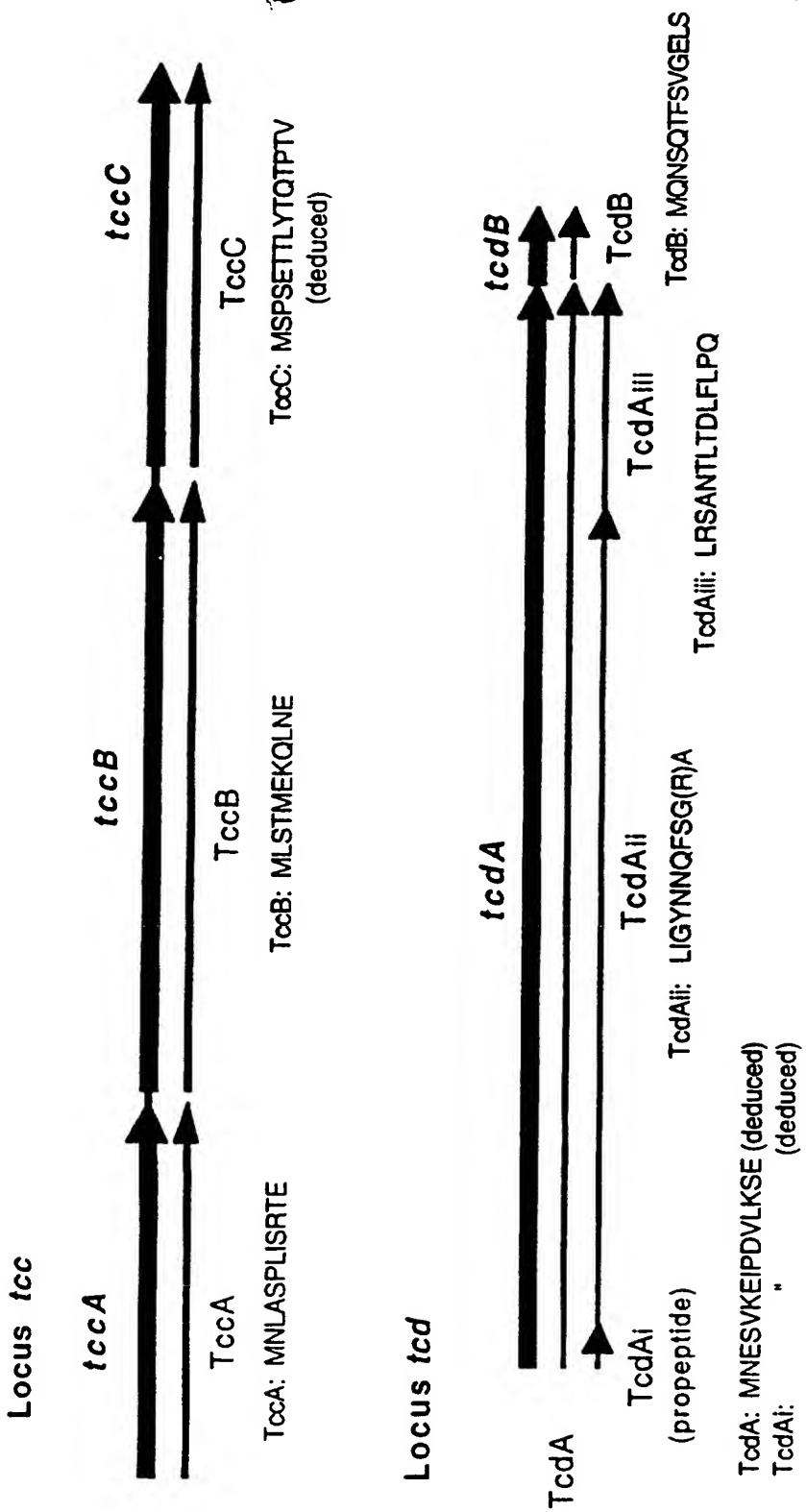


FIG. 6B

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18003

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :Please See Extra Sheet.
US CL :536/23.7, 24.1; 435/172.3, 240.4, 320.1; 800/205; 47/58
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.7, 24.1; 435/172.3, 240.4, 320.1; 800/205; 47/58

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CABA, CAPLUS, MEDLINE, GENBANK, BIOSIS
search terms: photorhabdus, xenorhabdus, luminescens, insecticide, nematode, lepidoptera

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | CLARKE et al. Virulence Mechanisms of *Photorhabdus* sp. Strain K122 toward Wax Moth Larvae. Journal of Invertebrate Pathology. 1995, Vol. 66, pages 149-155, see entire document. | 1-64 |
| Y | US 5,039,523 A (PAYNE ET AL.) 13 August 1991, columns 1-10. | 1-64 |
| Y | US 5,254,799 A (DE GREVE ET AL.) 19 October 1993, columns 1-14. | 1-64 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
23 DECEMBER 1996

Date of mailing of the international search report
28 JAN 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18003

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12N 5/14, 15/00, 15/05, 15/09, 15/29, 15/31, 15/64, 15/82; A01G 13/00; A01H 1/00

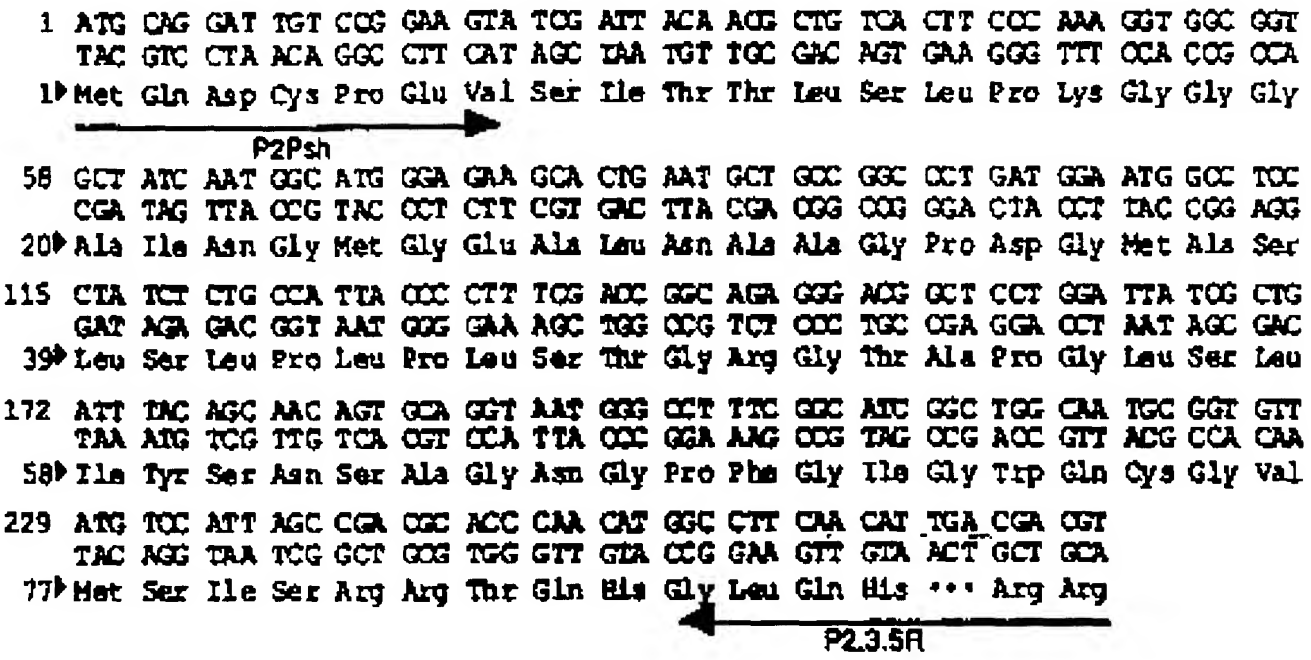



FIG. 1

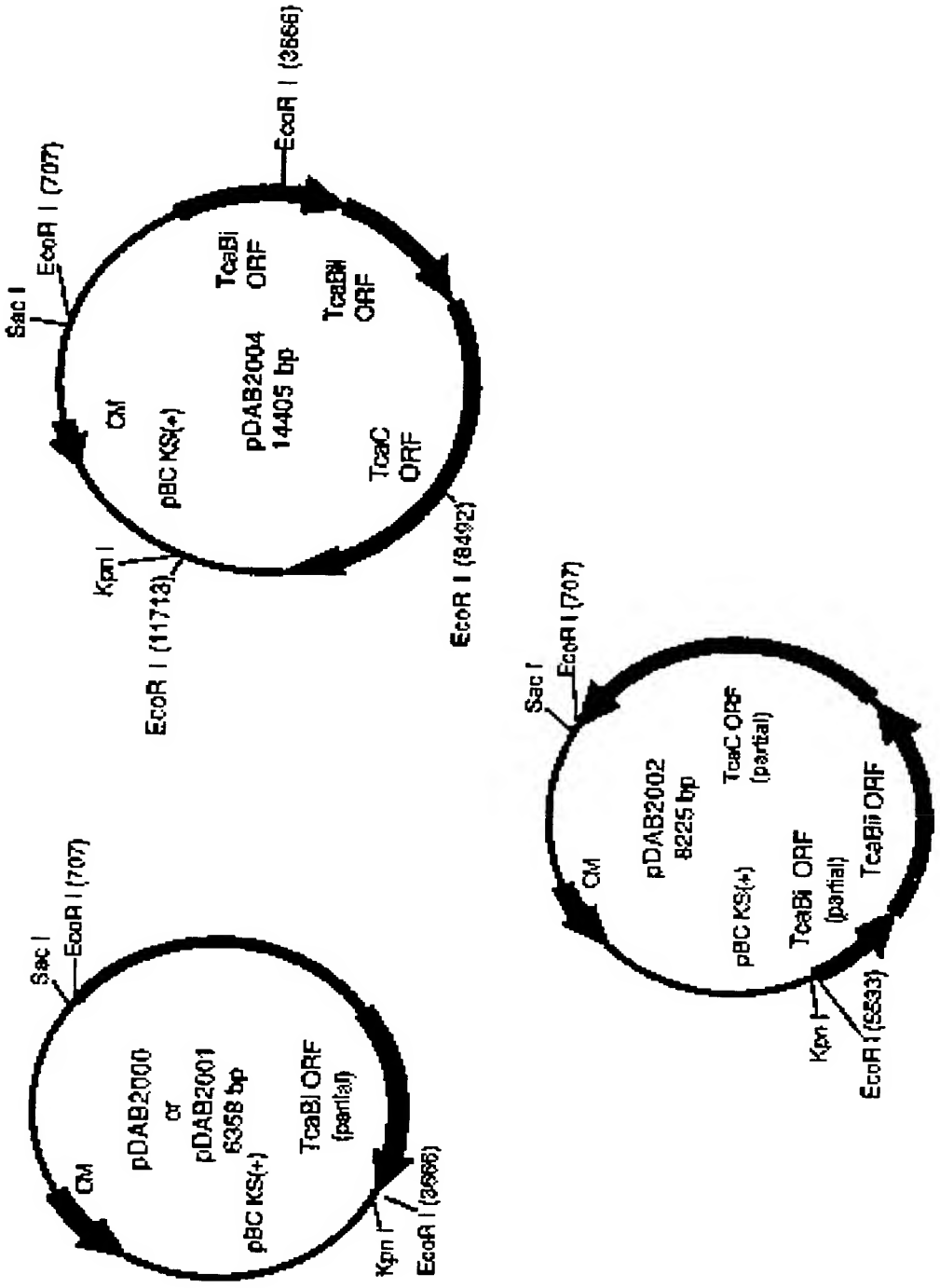



FIG. 2

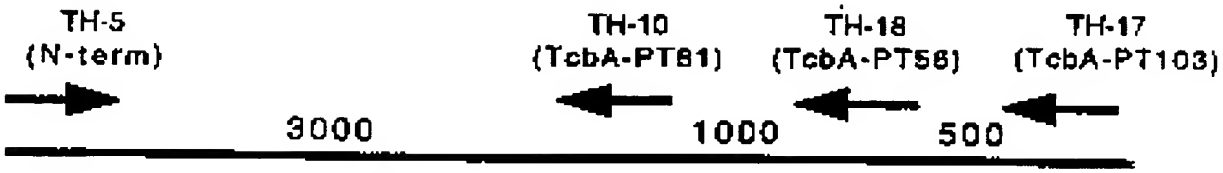



FIG. 3

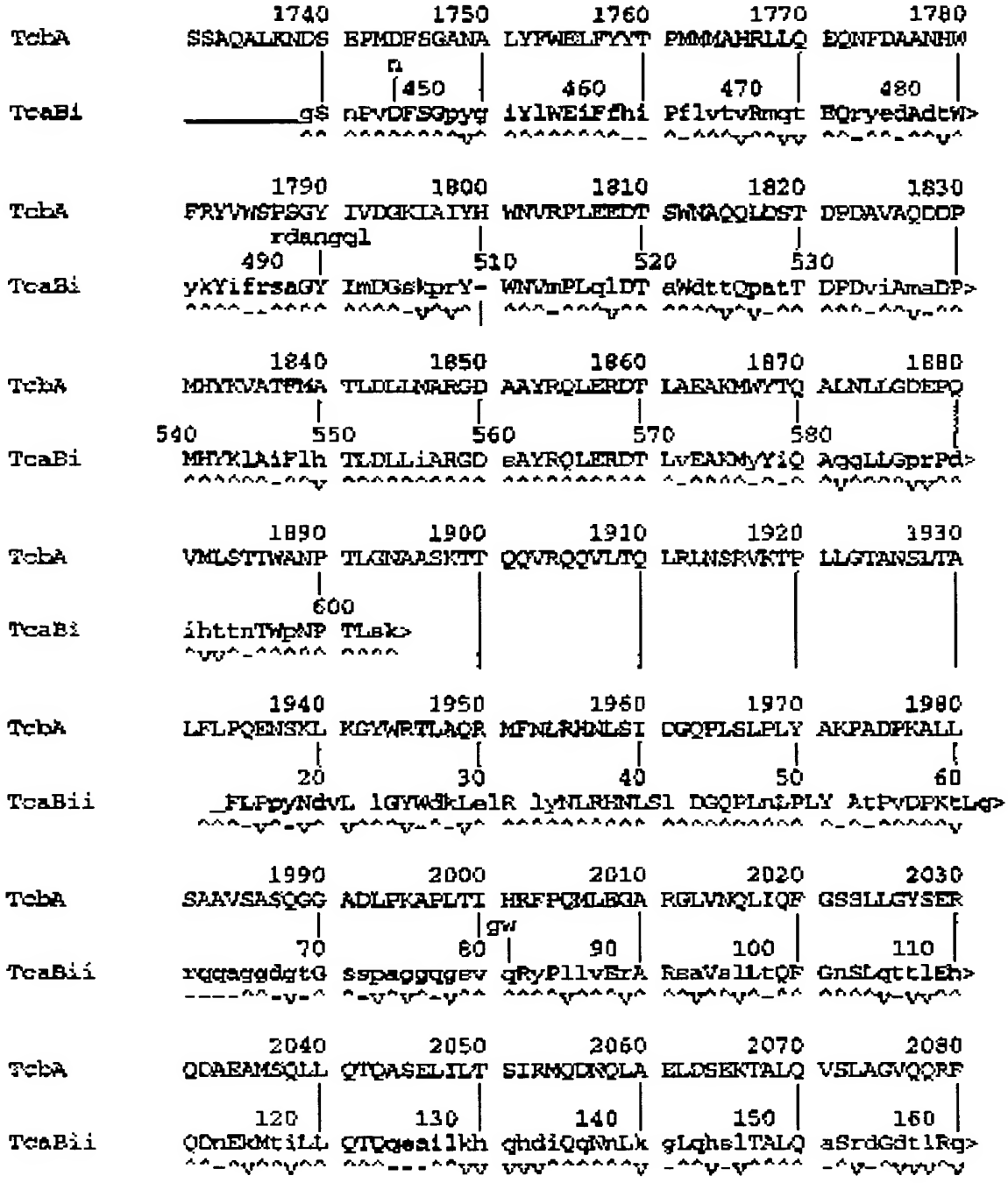



FIG. 4A

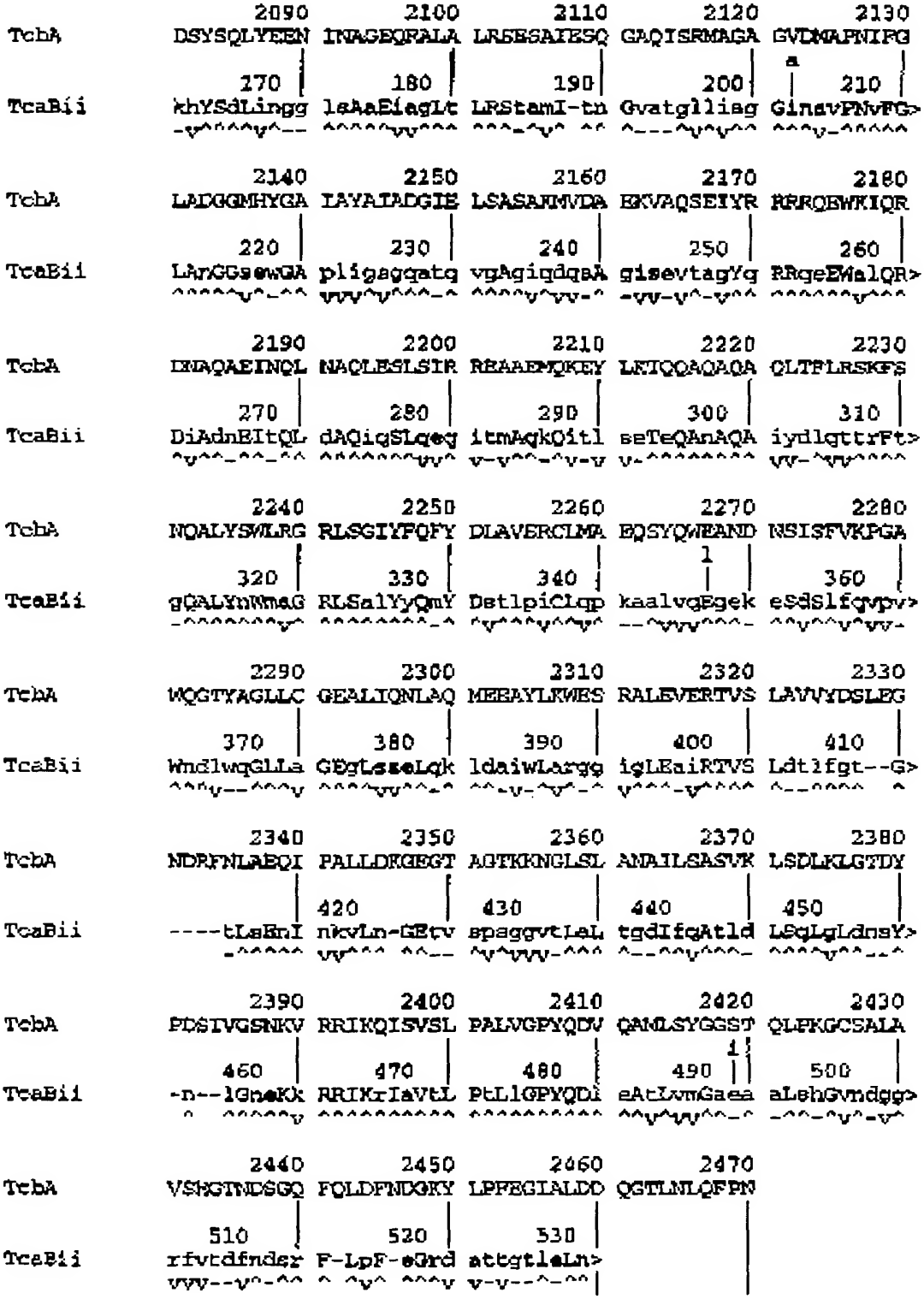



FIG. 4B

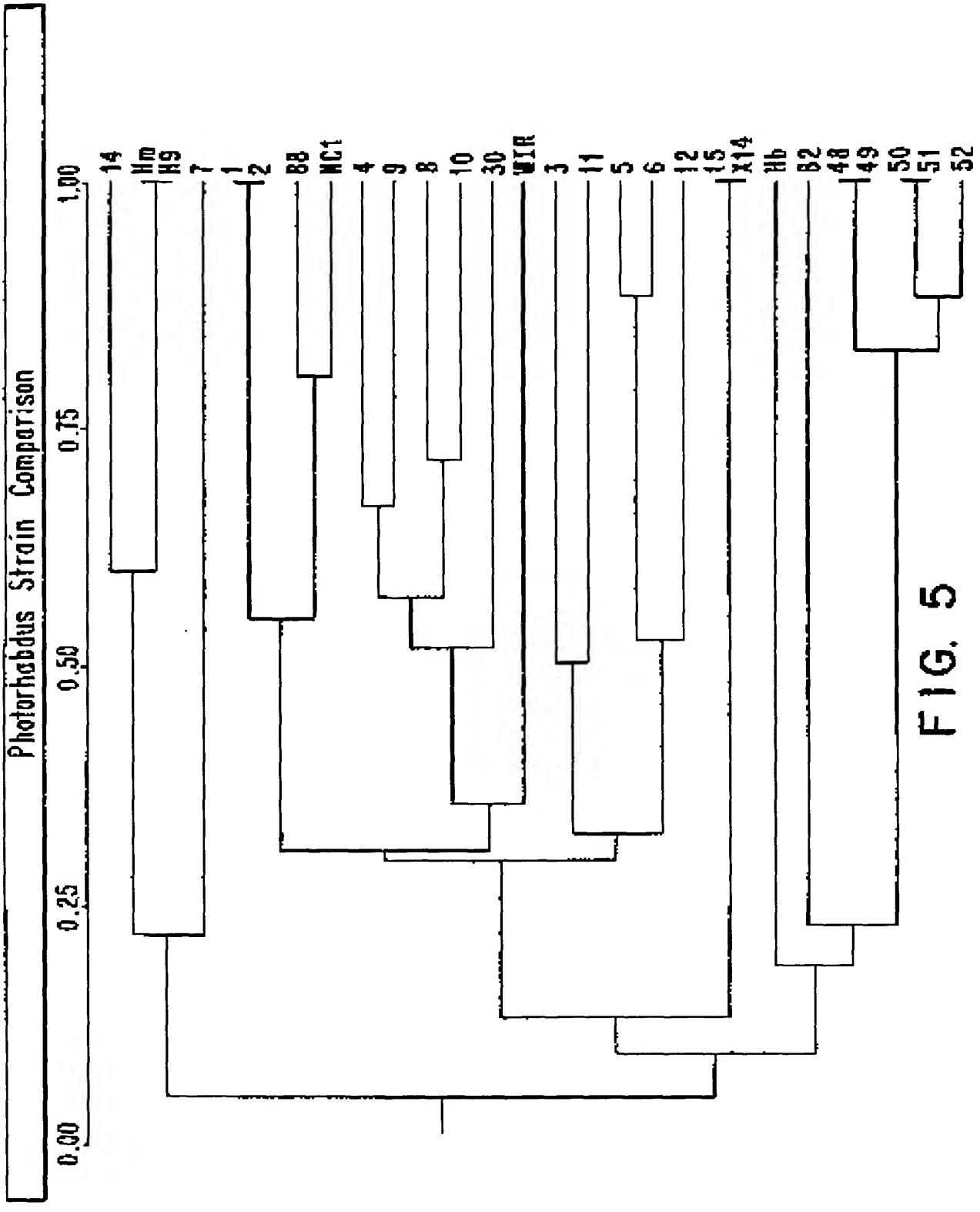
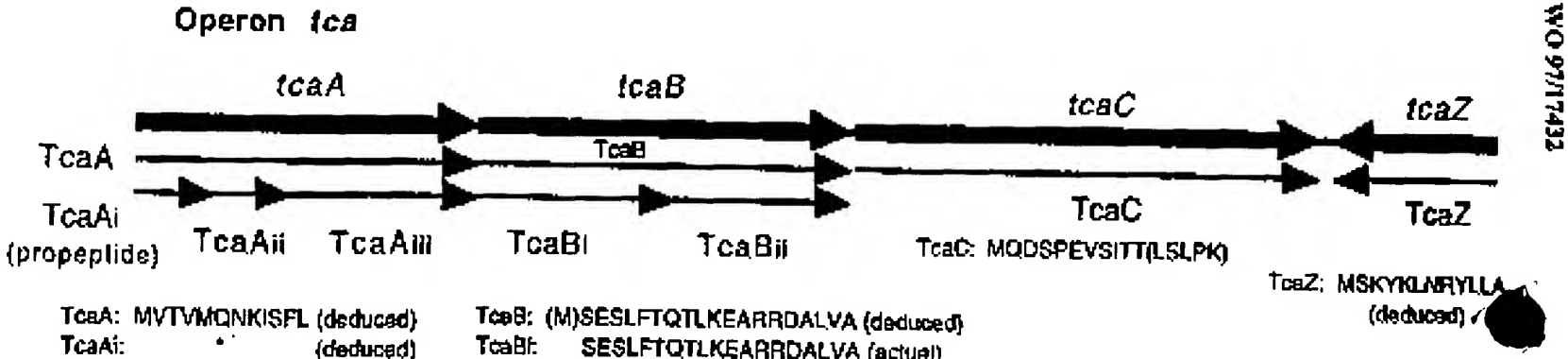



FIG. 5



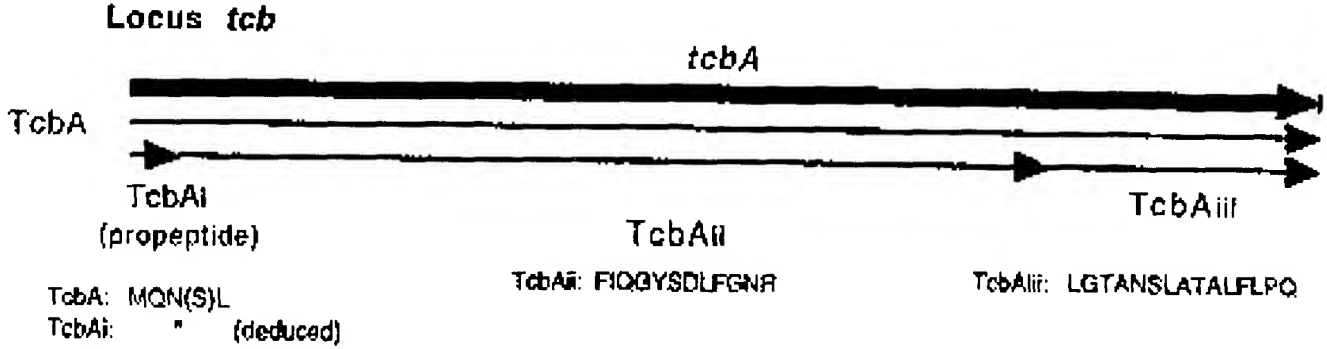




FIG. 6A

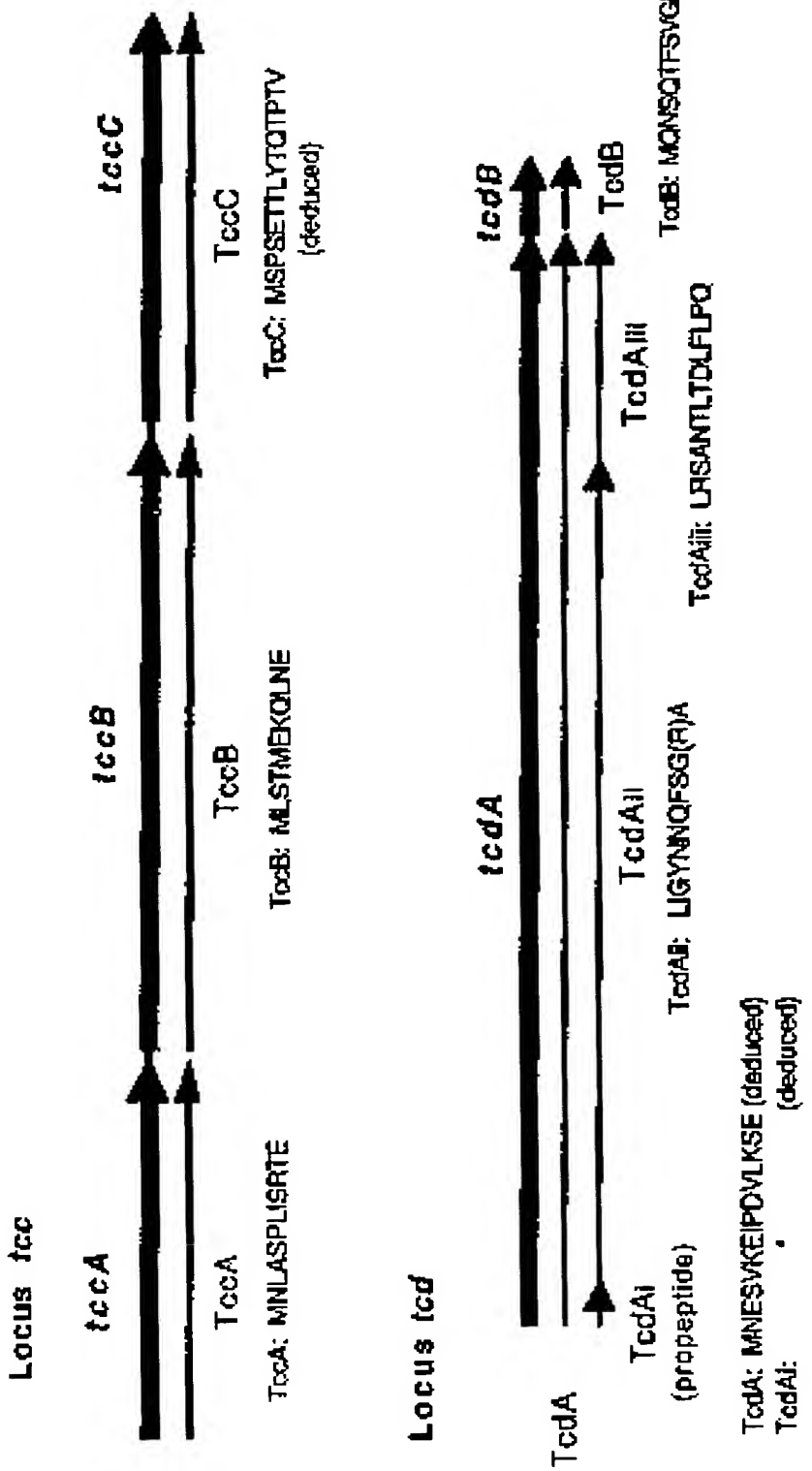



FIG. 6B